LEUCOCYTES AND THE REDUCTION OF METHYLENE BLUE IN MILK

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TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

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GENERAL INTRODUCTION

The methylene blue reduction test is one of the three standard methods of estimating bacterial populations in milk which are in universal use in milk control work. This test is used on the Calgary and Edmonton raw milk supplies almost to the entire exclusion of all other bacteriological tests.

Operators of the test have observed for many years that there are occasionally milks of short reduction times which do not contain sufficient numbers of bacteria as measured by the plate count or the microscopic count to account for the reduction by bacterial action. Some of these milks were known to be high in leucocyte contents and for twenty years it has been customary to accord to leucocytes a reducing power of sufficient magnitude to be a factor in the reduction of methylene blue in milk.

The present study which is an attempt to throw some light upon the reducing properties of leucocytes in milk, naturally divides itself into three phases which will be discussed in the following three sections:-

I. The accuracy of the available methods of measuring bacteria and leucocytes in milk.

II. The role of leucocytes in the reduction of methylene blue in milk.

III. The incidence of mastitis among the cows in the Edmonton milk shed.

METHODS

All samples of milk were obtained by the author during a study of milk production problems in the Edmonton district and are divided into three classes, viz.:-

1. Herd milk, which is mixed milk from one or more cows drawn into utensils of varying degrees of sterility the samples being taken from the cans.

2. Udder milks, which are composite samples of approximately equal amounts of milk from each milking quarter carefully drawn into sterile flasks from half empty udders which were in almost all cases previously washed with disinfectant solution.

3. Quarter milks, which are samples drawn from individual quarters using the technique described in '2'.

These samples were immediately brought to the laboratory and prepared for testing. The maximum time elapsing between sampling and the start of the test was three hours.

Standard Methods of Milk Analysis (1929) was followed for the plate counts, the microscopic counts, and the methylene blue reduction test, except that 1% of glucose was added to the standard nutrient agar used in the plating technique. The standard methylene blue test was supplemented by a modified technique (Thornton and Hastings, 1929) which

consisted of shaking the tubes of methylene blue milk each half hour during incubation in the constant temperature water bath. The tubes used in the standard methylene blue technique were plugged with non-absorbent cotton while rubber stoppers were employed in the case of the modified technique. The counting of plates was done with the aid of a lumi-lens colony counter and a hand tally was used for recording all bacterial and leucocyte counts.

The Breed factor of the microscope used in the microscopic technique was almost exactly 600,000. Microscopic counts of both bacteria and leucocytes are based upon the examination of a number of fields varying from 5 to 6000 fields (whole smear) per smear. Unless otherwise stated, the count is based upon the examination of 60 fields. All leucocytes, lymphocytes and epithelial cells are classed as leucocytes in this study.

Where the term "counts" is used it invariably means counts per cubic centimeter.

In this study the possibility of the presence of unstained and therefore uncountable leucocytes in the milk was recognized but was disregarded. The fact that different kinds of leucocytes may have different reduction abilities was also disregarded.

Reduction times are reported in hours and minutes: 9:30 meaning 9 hours and 30 minutes.

The following terms are used to mean as described below:-

"Chain" four or more bacterial cells occurring in chain formation.

"Clumps" two or more bacterial cells occurring close together in formations other than chains.

"Groups" one or more bacterial cells occurring as isolated individuals, chains or clumps.

P A R T I

THE ACCURACY OF THE AVAILABLE METHODS

OF MEASURING BACTERIA AND LEUCOCYTES

IN MILK

INTRODUCTION

A number of different methods of measuring bacteria in milk have been developed, but only three of these methods have retained a sufficient degree of popularity to be to-day in general use in milk control laboratories. These three methods are: the Standard Plate Method, the Direct Microscopic Method, and the Methylene Blue Reduction Test. These methods are recognized by the American Public Health Association in "Standard Methods of Milk Analysis".

The accuracies of these methods have been studied during the past few decades and a great deal of controversy between different workers has resulted. In many cases in the past the accuracy of these bacteriological methods has been studied by comparing bacterial counts obtained by the same method from duplicate samples. In such cases it is possible that these counts may be affected by a constant source of error which affects all duplicates nearly proportionately. It is reasonable to suppose that such an effect may be produced on replicate plate counts by the clumping of bacteria, and it may be possible to obtain reasonably comparable replicate counts by this method, while such counts may not necessarily be a true measure of the actual bacterial content of the milk. In the early studies

on the accuracy of the methylene blue reduction test many workers compared it with the plate method assuming on the basis of experiments of the nature cited above that the plate method is reasonably accurate, and the discrepancies which were frequently observed between the two methods were in many cases attributed to the inaccuracy of the methylene blue reduction test. However, the more recent work shows a tendency to question the accuracy of the plate method.

The experimental work on the accuracy of the direct microscopic method as it is applied to milk analysis has not been very extensive. This is perhaps partly due to the fact that this method is less frequently employed in milk control work.

In this section of this report an attempt will be made to illustrate with experimental data some of the reasons for these methods tending to give conflicting results so frequently. Particular attention will be drawn to the methylene blue reduction test and its relationships to the other bacteriological tests.

Since the main reason for this study is to learn the effect of leucocytes in the methylene blue reduction test, and since recent work on the occurrence of leucocytes in milk has been very extensive, it was thought advisable to study the accuracy of the direct microscopic method

(Prescott and Breed (1911)) which is to-day almost universally used in estimating the leucocyte content in milk. Data will be presented which will bring to light some new information on the accuracy of this method as it is applied in the quantitative studies of leucocytes in milk.

PLATE COUNTS AND METHYLENE BLUE REDUCTION TIMES OF HERD MILKS

During the course of our study of milk contamination in this district it was frequently observed that many samples of herd milk and of udder milk gave very low plate counts and unexpectedly short reduction times. A number of such milks are reported in Table I. A similar relationship has been reported by Dorner (1933). This worker found that of 531 samples of milk with bacterial contents of less than 50,000 per c.c. as shown by the Burri technique, 129 had reduction times of less than six hours. Miles (1933) also reported discrepancies of the same nature, and on the basis of such results this worker comes to the conclusion that the only advantages the methylene blue reduction test has over the plate method are its cheapness and the comparative rapidity and ease with which the test can be carried out. According to this worker's claim, the reductase test as compared with the plate method is not a fair method for

the grading of milk intended for liquid consumption. Miles also states that he found a closer correlation between the plate counts and the keeping quality than between the reduction time and the keeping time. His data, however, do not show this very clearly. While it is true that this investigator shows samples of milk among his data which reduced in less than two and one half hours and remained sweet for three days, this does not necessarily prove the inaccuracy of the reduction test. It may be that some of these milks came from abnormal udders and were reduced not as a result of bacterial activity but due to causes other than bacteria. This worker also reports milks with low plate counts and exceptionally short reduction times. If such discrepancies are attributed to the inaccuracy of the reduction test then it seems that this worker failed to take into consideration the clumping of bacteria in milk, and also the effects of clumping on the plate count and the reduction time. It is obvious that the plate method does not measure the individual cells within the clump while on the other hand it is probable that the reduction test measures the activity of all active bacteria present in the sample of milk. This, therefore, indicates that it may be the plate method which is at fault rather than the reduction test.

In the past it has been usual to attribute such discrepancies to the inaccuracies of the reduction test.

Due to the work of Skar (1913, 1931) leucocytes have been viewed with suspicion in connection with their reducing properties in milk, and some workers attribute some of the pronounced discrepancies between plate counts and reduction times to the action of leucocytes in the reduction test. An attempt will be made in this paper to discuss this question and data will be presented which will throw some light on both of the above contentions.

In examining the figures reported in Table I, we find many extreme and unexpected results. It will be noted that of the 56 milks reported, 18 gave plate counts of less than 50,000 and reduction times of less than six hours. We should also note that two of these milks reduced in less than two and one-half hours and gave plate counts of not more than 500 per c.c.

Data reported in Table II show the existence of marked differences between the reduction times of milks produced in sterile utensils and the reduction times of milks produced in producer's utensils. These differences are more pronounced in the modified reduction times than they are in the standard times. On the other hand no such apparent differences are shown by the plate counts of these milks. The plate counts of producer's utensil milks show in the majority of cases insufficient bacteria to account for the respective reduction times. Where keeping times were studied it was found that in every case sterile utensil milk remained sweet much longer than did the milk that was

produced in producer's utensils. The plate counts and the methylene blue reduction times of the different milks produced on these ten farms are averaged in Table II. The average standard reduction time for the sterile utensil milks was 12:20 as contrasted with an average reduction time of 8:16 for producer's utensil milk. The average plate count of sterile utensil milks was 1,170 as compared with 8,538 for producer's utensil milks. We should note that the average plate count of the latter milks would be less than 4,000 if Milk "C" from farm No. 2 were excluded. These data show very clearly that it would not be fair to judge the bacteriological qualities of these milks on the basis of the plate count. These observations prove that bacteria must have been present in most of these milks, which the methylene blue reduction test measured more adequately than did the plate count. Therefore, it was suspected that clumping of bacteria was responsible for the discrepancies noted, since it is universally recognized that bacteria, particularily those from non-sterile utensils, tend to exist in large groups.

Data reported in Table III will show that many of these herd milks harboured large numbers of groups of bacteria. This is shown by the difference between group counts and individual counts. Since these microscopic counts are based on the examination of 60 fields per smear, we do not consider them very accurate. To substantiate the results given by the microscopic examinations, smears were made from nineteen of these milks at the moment of reduction. All of these smears

were made from the tubes in the modified tests. If reduction of the methylene blue in milk is the result of bacterial activity then a microscopic examination of the milk at the moment of reduction should reveal sufficient bacteria to account for that phenomenon. The bacterial counts at the moment of reduction of the nineteen herd milks reported in Table III show that in every case there were enough bacteria present to account for the reduction times of these milks. The accuracy of these counts is somewhat questionable as they are based on the examination of only five fields per smear, and it was found difficult to count the individual cells with extreme certainty due to the presence of large clumps and chains. These figures should, therefore, be considered only as estimates. It is believed that these estimates are reasonably accurate and in any case likely represent the minimum number of bacteria present. The average bacterial count at the moment of reduction of these nineteen milks is 108,324,000 per c.c., which is much higher than the average plate count of milk at the moment of reduction reported by Thornton and Hastings (1929). These workers reported an average plate count of approximately 21 million. It is reasonable to expect plate counts to be considerably lower than microscopic counts due principally to the clumping of bacteria.

On closer analysis of the data presented in Table III, we find ten milks which showed plate counts of less than

50,000 and which had reduction times of less than six hours. These plate counts, therefore, seem to indicate that there were insufficient bacteria to account for these reduction times. When we examine the microscopic counts of these milks, we find that large groups of bacteria were found in all cases, and in the case of those where smears were made at the moment of reduction and examined microscopically, enough bacteria were observed to account for the reduction. The microscopic counts of individual cells alone show sufficient bacteria to account for the reduction times. If we were to assume a generation time of one-half to one hour for the bacteria during incubation of the methylene blue tests, we would find that the microscopic count of the individual cells would give an arbitrary count at the moment of reduction which would approximate the average of the 19 counts made at the moment of reduction as reported in Table III. On the other hand it is evident that the plate counts reported in Table III will not in the majority of cases give similar theoretical results. In Table VIII microscopic counts are reported where 1,000 fields per smear were examined. These data also show great variations between the bacterial plate counts and direct microscopic counts. The following milks are the more outstanding in this respect, namely, milks Nos. 8, 17, 23, 39 , 60, 68 and 70. It will be noted that large groups of bacteria were observed in all these milks. Upon noting the reduction times of these milks, we find that in no case these exceeded 9:30. Since these milks reduced in

comparatively short times and large groups of bacteria were observed, it may be concluded that the bacterial counts obtained by the plate method were far from being accurate.

On the basis of these data we may conclude that the plate counts are often misleading as they do not measure the true bacterial content of milk when bacteria tend to occur in large aggregates. On the other hand, these results seem to show that the methylene blue reduction test measures the bacterial content of such milks much more accurately. This therefore, indicates that the reduction test is not affected by clumping of bacteria to the same degree as is the plate count.

THE ACCURACY OF THE MICROSCOPIC COUNT OF BACTERIA IN MILK

The accuracy of the direct microscopic method as it is applied to the measuring of bacterial populations in milk has been studied by various workers. The most extensive study that appears in the literature is reported by Breed and Stocking (1920). These workers investigated the accuracy of this method by comparing the counts on duplicate smears obtained by two analysts. The counts were based on the examination of 100 fields per smear. The data reported by these investigators show that they were able to obtain counts that

checked rather closely. On the average it may be said that their results checked closer than one would expect when all factors that tend to affect this test are taken into consideration. These workers also compared the microscopic counts with the plate counts. Their results show that in most cases the plate count comes between the microscopic count of groups (isolated single cells, chains and clumps) and the microscopic count of individual cells.

In the study reported here an attempt was made to gather some information on the extent of clumping of bacteria in milk and also on the distribution of clumps, chains and bacterial cells throughout the smear. It has not been infrequently observed in the case of milks giving short methylene blue reduction times that there may appear very few or no bacteria when only 60 fields per smear are examined microscopically which would indicate insufficient bacteria to account for the reduction. We may question the accuracy of the microscopic counts particularly in the case of milks with low bacterial content for the following reasons:

1. Where the bacteria, especially small cocci, occur singly, it is frequently impossible to distinguish them with certainty from stained, non-bacterial matter. This factor becomes less important in milks where bacteria are present as diplococci, chains or as clumps.

2. The uneven distribution of the bacteria in the milk and the small number of bacteria in the entire smear. An entire smear (0.01 c.c. of milk) would contain only 100 bacterial cells if the original milk contained 10,000 bacterial cells per c.c.. If there were approximately 6,000 microscopic fields per smear then in the example cited there would be an average of only one bacterium per sixty fields. The normal uneven distribution of these bacteria is exaggerated because of clumping and therefore in this study it was not infrequently found that many times sixty fields had to be examined before an organism was encountered. This is illustrated in the figures of the 37 udder milks reported in Table IV.

An examination of these data reveals a few significant things. In the first place it should be noted that in 22 of these milks no bacteria were found when only sixty fields per smear were examined. In 13 of the 22 milks, groups ranging from two to 1150 cells were found when 1,000 fields were examined. We should also note that with milks containing less than 35,000 bacteria per c.c. as determined by the examination of 1,000 fields per smear, there were no bacteria encountered when only sixty fields per smear were searched. This, however, does not mean that no bacteria would ever be found in such milks when only sixty fields per smear are searched, but nevertheless this seems to indicate that there is only a small chance of encountering bacteria

in such milks when employing this microscopic technique.

Samples Nos. 5 and 20 should be noted: these samples gave counts of 107,400 and 720,000 bacteria per c.c. respectively when 1,000 fields were examined, but no bacteria were found when only sixty fields were searched. In both of these milks large groups were observed. We should note, too, that in the case of 12 milks, more than 100 fields had to be examined before any bacteria were encountered. The 1,000 field counts of these 12 milks ranged from 4,200 to 36,000 per c.c., and the largest group found in any one of these milks was a clump of 6 cells. This, therefore, is indicative that uneven distribution of cells throughout the entire smear is not confined to very low count milks and those containing clumps and chains, but occurs also in milks containing relatively large numbers of bacteria existing as single and diplococci.

If we compare the 60 and the 1,000 field counts, we find that in the majority of low count milks the examination of 60 fields per smear reveals the absence of bacteria, while with milks relatively low in bacterial content but containing large groups of bacteria, a very high count may be obtained when only 60 fields are examined. This is illustrated by Samples Nos. 18 and 24. These figures seem to show definitely that a microscopic count based on the examination of sixty fields per smear may be

very misleading, and does not invariably tell anything about the true bacterial content of milks that harbour relatively few bacteria, and of those milks where large groups of bacteria are found.

In these data there are 9 milks reported of which 2,000 fields per smear were examined. If we compare the 2,000 field counts with the 1,000 field counts, we find in most cases rather wide variations between these figures. Samples Nos. 12 and 33 are the most significant in this regard. It will be noted that in both these milks large groups of bacteria were found. It appears that these variations are due entirely to the uneven distribution of chains and clumps throughout the entire smear.

On the basis of these experimental results, we may conclude that the inaccuracies in the direct microscopic method of estimating bacteria in milk are mainly due to the uneven distribution of single cocci and of clumps and chains of bacteria in the stained smear. These results further show that this method may give misleading results particularly when used on low count milks and on milks that harbour many bacteria in the form of large groups.

THE ABILITY OF THE 0.01 C.C. PIPETTE (BREED) TO DELIVER A REPRESENTATIVE SAMPLE OF MILK FOR THE SMEAR

Breed and Brew (1916) presented some data on the accuracy of the standardized 0.01 c.c. pipette. They weighed the amounts of milk discharged by the pipette and obtained an average weight of 0.0101 gram for 20 discharges. These workers reported 2.0 per cent as the maximum deviation from the mean for the accuracy of the pipette in measuring samples of milk for the smear.

To substantiate the findings reported in Tables III and IV on the uneven distribution of bacteria over the entire smear, two series of 8 replicate smears in each series from two samples of aseptically drawn milk were subjected to a microscopic examination in which 6,000 fields (entire smear) were examined. Data presented in Table V show the results of this study. The samples were kept thoroughly mixed during the preparation of the smears. All smears were made of discharges from the same pipette which was thoroughly cleaned and dried after each discharge. The smears were all examined with utmost care under the microscope and in every case the total number of bacteria occuring in the entire smear was recorded. An ocular micrometer for the Breed technique was used with the regular standardized microscope, and

strips of the smear equal in width to the diameter of the circle on the ocular micrometer were examined. The first strip was started in the corner of the smear and the slide was moved slowly until the other side of the smear was reached. Then from here a new strip lying parallel and closely adjoining the first was examined in the same manner as the first. This process was continued until the whole smear was covered. In this way (by using the ocular micrometer) only the central part of the actual field was examined, which portion of the field appeared in a fairly uniform focus. It was, however, found necessary to focus the objective frequently in order to differentiate between bacteria and other stained matter.

The figures reported in Table V seem to show quite definitely that the small portion of the milk drawn for the preparation of Breed smears is not always representative of the sample of milk studied. Data pertaining to milk No. 1 show that the variations are mostly due to clumping of bacteria. This is shown by the fact that variations in the counts of individual cells are much higher than in the counts of groups. The relatively low variations in the group counts seem to show that these figures probably represent mostly bacterialcells and not stained non-bacterial matter. The most significantthing about these data is the fact that in some of the smears hardly any large groups were found, while in others large

groups were encountered. It is not likely that the large groups of bacteria were missed during the examination as the smears were in all cases subjected to an equally thorough examination. The fact that the group counts are as uniform as they are shows that the smears were thoroughly covered. The uniformity in the counts of groups seems to indicate that the 0.01 c.c. pipette when properly cleaned discharges uniform amounts of milk.

We should also note that the average microscopic count of individual cells in sample No. 1 is 5.4 times as high as the plate count of this sample, while the highest microscopic count is more than 10 times as high as the plate count of the same sample. Duplication in the microscopic counting may have been a factor in this work, but was likely quite small, as this was guarded against as much as was possible.

We may conclude from the data reported in Tables III, IV and V that the inaccuracies of the direct microscopic method when used in the analysis of the classes of raw milk under discussion, are mainly due to the uneven distribution of bacterial cells and of groups of bacteria throughout the entire smear, and also to the fact that the 0.01 c.c. pipette does not remove a representative sample of milk for the preparation of smears.

It should be noted that in this discussion no account was taken of dead bacteria and of bacterial cells

which may not have taken the stain. These factors may be important when making direct comparisons between the results obtained by this method and those obtained by some other method, but in a study of this nature these factors seem to appear less significant. It is possible that any error contributed by the inability of certain bacteria to take up the stain would be of a more or less constant nature and would affect the replicate counts equally.

In the discussion heretofore, it has been proven that with herd milks which give low plate counts, low routine microscopic counts and short reduction times, and which show large groups of bacteria by the extensive microscopic examination (1,000 fields per smear) and also enough bacteria at the moment of reduction to account for the reduction, the methylene blue reduction test measures the bacterial populations far more accurately than does either the plate method or the routine microscopic method. Furthermore, these data seem to show that the latter two methods often give misleading results with respect to bacterial populations in such milks.

We have seen that clumping of bacteria is the main factor causing such discrepancies in the results obtained by the three different methods. It has been shown that large groups of bacteria are not uniformily distributed throughout the milk and that in the microscopic technique

it may not always be possible to obtain a representative sample of milk for the smears. Also since bacteria are not uniformily distributed throughout the smear it is impossible to secure accurate information on the bacterial content of such milk by the routine microscopic examination. The inaccuracy of the plate method due to clumping of bacteria is self-explanatory and need not be further discussed here. On the other hand the reduction test does not seem to be affected by clumping of bacteria in the same way and therefore this test probably measures the bacterial populations of such milks much more accurately than does the plate method.

So far we have only dealt with milks where enough bacteria were found to be present to account for the reduction of the methylene blue in the reduction test. We are yet to deal with milks which were found to give short reduction times, low plate counts and low microscopic counts, and in which insufficient bacteria were found at the moment of reduction to account for that phenomenon. This, therefore, leads us to the study of the role of leucocytes in the reduction test. This subject will be discussed in another section of this paper.

THE ACCURACY OF THE DIRECT MICROSCOPIC METHOD IN ESTIMATING THE LEUCOCYTE CONTENT OF MILK

Since the work already reported has shown that the direct microscopic method may give misleading results on the bacterial content of milk and since the quantitative aspects of leucocytes in milk are receiving a great deal of attention at the present time, and also since our study centers around the leucocyte contents of milk and their relationship to the methylene blue reduction test, it was thought advisable to gather some data on the accuracy of the direct microscopic method of estimating the leucocyte content of milk.

A search in the literature showed only a few instances where studieshave been reported on the accuracy of the direct microscopic method (Prescott and Breed, 1911) of estimating leucocytes in milk. It should be noted here that this method is in universal use at the present time for the enumeration of leucocytes in milk.

Breed and Stidger (1911) demonstrated that by the use of this technique many more leucocytes were present in normal milk than had been previously thought to occur.

The accuracy of this method has been studied by Prescott and Breed (1911). They made a series of 31 tests in duplicate -- counting 100 fields per smear, and found a variation of 14.5 per cent. However, they reported two

cases where the variation were 42.9 and 64.3 per cent respectively. In each case the leucocyte content was less than 250,000 per c.c.. According to the findings reported by these workers, the percentage variations tend to be greater with milks low in leucocyte contents. These investigators concluded that the variations seem to be due to inaccuracy in the counting rather than to the inaccuracy in the preparation of the smears. Even on the same smear a difference of 15 per cent may occur in two different counts according to their report. Since these workers did not present complete data and did not state by what method they calculated the variations it is difficult to appreciate the full significance of their figures.

Some workers are of the opinion that the leucocyte counts are not accurate because of the tendency for leucocytes to appear in clumps. During the study reported here the observation in this regard has been that this seems to be true with milks exceptionally high in leucocytes, but in such cases it seems that clumping does not contribute very much towards the variations in the counts when as many as 60 fields per smear are searched. With milks containing roughly less than one million leucocytes per c.c. clumping does not appear to be very prevalent. The inaccuracies of this method seem to be due mainly to the uneven distribution of leucocytes over the entire smear. The data reported in Table VII will possibly give some support to this contention.

The data reported in Table VI give leucocyte counts of replicate smears made from four samples of milk. Each count reported is based on the examination of 60 fields per smear. The variations are expressed in three ways. It will be noted that the maximum deviation from the mean varied from 25.05 per cent to 42.07 per cent, while the average maximum deviation from the mean for the four milks was 30.92 per cent. On the other hand, the average absolute deviations from the mean are fairly uniform for the four milks. These varied from 12.11 per cent to 17.14 per cent while the average for the four milks was 14.03 per cent. These data reveal that the direct microscopic method of estimating the leucocyte content of milk is subject to a greater degree of inaccuracy than has been anticipated.

A Breed smear from each of the 7 milks reported in Table VII was examined by the following technique: 720 fields per smear were observed and the number of leucocytes in each field noted. Per c.c. counts were then computed for each 60 fields in the order of observation giving a total of 12 such counts per smear. Throughout this work an attempt was made to prevent duplication in the examinations asmuch as was possible.

Scrutiny of these data gives some idea of the distribution of leucocytes throughout the smear. The average maximum deviation from the mean for the 7 milks

was 44.24 per cent. The average absolute deviation from themean varied from 9.34 to 29.03 per cent with an average of 14.99 per cent for the 7 milks.

In view of the fact that data reported in Tables VI and VII show high variations, it is evident that these variations were not due to the inaccuracies in obtaining a sample of milk for the smear, but were due to the uneven distribution of the leucocytes throughout the smear. This contention is supported by the fact that variations were as high among the counts obtained from the same smear as among thoseobtained from the different smears of the same milk.

These data also seem to confirm the findings of other workers who have reported greater variations with milks low in leucocyte content. When we separate the milks reported in Tables VI and VII that gave average leucocyte counts of more than one million, from those that showed less than this number of leucocytes per c.c., we find that the average maximum deviation from the mean for the former class of milks is 30.14 per cent, and for the latter class it is 39.16 per cent. The average absolute deviation from the mean is 12.83 per cent and 16.15 per cent for the respective classes. However, it should be noted that the figures for the latter class of milks are influenced considerably by milk No. 4 in Table VII. When this milk is omitted, the average deviation from the mean for milks with leucocyte

counts of less than one million is 33.73 per cent, while the average absolute deviation from the mean comes down to only 13.58 per cent. These figures, therefore, only tend to show that variations seem to be higher with milks relatively low in leucocyte content than with milks containing large numbers of leucocytes.

In view of the ratios of the lowest counts to the highest counts reported in Tables VI and VII, it appears that this method may often give misleading results on the actual leucocyte populations of milk. The average ratio of the lowest counts to the highest counts for the 11 milks is 1:1.90. Therefore it is evident that the highest count may be expected to be approximately 200 per cent of the lowest count. We judge this to constitute reasonable accuracy in the routine estimation of leucocytes in milk.

P A R T II

THE ROLE OF LEUCOCYTES IN THE REDUCTION

OF METHYLENE BLUE IN MILK

INTRODUCTION

There has been very little work reported on the effect of leucocytes on the methylene blue reduction test. Skar (1913, 1931) seems to have given this question the most consideration. In his reports he proves to his own satisfaction that leucocytes in milk exhibit very marked reducing properties. However, he did not in any of his papers attempt to explain the mechanism of the action of leucocytes on the methylene blue. As far as research literature shows there seems to be no adequate explanation for this phenomenon.

In this paper an attempt will be made to show whether there is any relationship between the leucocyte content of milk and the methylene blue reduction time. Data will be presented relative to the study of relationships between the leucocyte content and the bacterial content of a number of udder milks. Also the bacteriological condition of milks drawn aseptically from udders which showed symptoms of abnormality will be dealt with in this study.

THE LEUCOCYTE CONTENT OF MILK AND THE PRESENCE OF CHAINS AND CLUMPS OF BACTERIA AS DETERMINED BY AN EXTENSIVE MICROSCOPIC EXAMINATION

In this work aseptically drawn milks from 95 cows were studied. In each case (with two exceptions) 1,000 fields per smear were examined microscopically. With regard to milks Nos. 1 and 2 only 5 and 200 fields per smear were examined respectively. It will be noted that the bacterial counts were high for these milks and therefore many bacteria were encountered on examination only of a few fields per smear.

Table VIII contains information on the maximum size of chains and clumps encountered in each milk. An examination of these data shows that 64 (67.3 per cent) of the 95 milks each contained more than 500,000 leucocytes. The remaining 31 (32.6 per cent) milks harboured 500,000 or less leucocytes per c.c. Clumps or chains of bacteria were found in 40 (61.5 per cent) of the milks containing over half a million leucocytes, while on the other hand in only 8 (25.8 per cent) of the 31 milks each containing less than 500,000 leucocytes were clumps or chains of bacteria found. It should also be noted that in 20 (21.0 per cent) of all the milks studied only single cocci were observed, and in 27 (28.4 per cent) both single cocci and diplococci were observed. Therefore, in 47 (49.5 per cent) of the 95 milks studied no clumps or chains larger than two cells

were found, while in the remaining 48 (50.5 per cent) milks clumps or chains were observed.

Of the 64 milks containing over half a million leucocytes per c.c., 29 (45.3 per cent) were found to harbour longchain streptococci when 1,000 fields per smear were searched. When we compare these results with the results reported in Table XV where of the 80 milks containing over half a million leucocytes per c.c., 16 (20.0 per cent) were found to harbour longchain streptococci as determined by the microscopic examination of 60 fields per smear, we find that these results show quite conclusively that by a microscopic examination of 1,000 fields per smear it is possible to detect the presence of longchain streptococci in milk far more accurately than by an examination of only 60 fields per smear.

It should also be noted that of the 48 milks in which clumps or chains of bacteria were found, 32 (66.6 per cent) reduced methylene blue in less than 10:00 by the standard technique. On the other hand of the 40 milks which gave standard reduction times of less than 10:00 only 8 (20.0 per cent) did not contain clumps or chains of bacteria. The leucocyte content of these 8 milks varied from 180,000 to 15,960,000 per c.c. and the microscopic counts of bacteria varied from 6,000 to 51,000 per c.c. It seems evident here that in some of these milks there were insufficient bacteria and leucocytes to account for the respective reduction times.

No rod shaped bacteria were observed in any of the

udder milks reported in Table VIII.

These data show that there is a greater tendency for clumping of bacteria in milks containing large numbers of leucocytes than is the case with milks harbouring less than 500,000 leucocytes per c.c. Most of the large groups of bacteria observed were in the form of longchain streptococci. This therefore seems to be in conformity with the findings of other workers who report a relationship between the appearance of large numbers of leucocytes in milk and the incidence of udder infections.

THE LEUCOCYTE AND BACTERIAL COUNTS OF MILK

The major part of the work reported in the literature on this subject is based on the bacterial counts obtained by the cultural plate method, while practically no attempt has been made to correlate the leucocyte counts with the bacterial counts obtained by the direct microscopic method.

Cherrington, et. al. (1933) report plate counts of milk using two kinds of media, namely plain agar and blood agar. Their results on milk from healthy cows show an average plate count of 1,000 on plain agar and an average

of 1,600 on blood agar. The leucocyte counts of these milks varied from 20,000 to 132,000. The milks from diseased cows showed bacterial averages of 15,000 and 40,000 on plain agar and blood agar respectively. The leucocyte contents of these milks varied from 21,000 to 13,640,000 per c.c..

Copeland and Olsen (1926) reported bacterial plate counts and leucocyte counts of milk from 40 cows. Their report reveals that in cases where leucocyte contents are over one million, the bacterial counts are usually high. An average plate count of 5,407 for such milks was reported by these workers.

Baker and Breed (1920) found that milks containing large numbers of leucocytes usually though not invariably contain streptococci. These workers, however, did not report the relationship of bacterial to leucocyte counts on a quantitative basis.

Breed (1929) states that numbers of leucocytes in milk may also be influenced by physiological disturbances in the milk secretion having no connection whatever with bacterial infection.

The work reviewed above is indicative of some relationship between the leucocyte contents and the bacterial contents of milk.

The graph in Figure I which is representative of Table VIII shows the standard methylene blue reduction times

the logarithms of leucocyte counts, and the logarithms of bacterial counts obtained by the direct microscopic examination of 1,000 fields per smear of each milk. It will be noted that the reduction times beyond 13:00 are not recorded in figure I. In plotting this graph the milks were arranged in the order of increasing bacterial counts. For this reason the bacterial count curve is fairly smooth while the other two curves are comparatively irregular. This should, therefore, be kept in mind when examining and comparing the three curves.

An examination of the logarithmic curves of leucocyte counts and bacterial counts shows some degree of relationship between the two curves. There is a general rise in the leucocyte count curve with the rise of the bacterial count curve. This relationship is quite apparent throughout the whole lengths of these curves.

In view of the work reported in the first section of this paper, we may reasonably expect these microscopic counts to be closer to the real or actual bacterial contents of these milks than the counts obtained by the plate method, and consequently the above relationship between bacterial and leucocyte counts may presumably be of greater significance than that which may be obtained when using plate counts for the same purpose.

It will be noted that there are fewer milks containing leucocytes in excess of 500,000 towards the lower

end of the bacterial count curve. While on the other hand towards the high end of this curve the majority of milks contained more than 500,000 leucocytes per c.c.. The average bacterial count for milks containing less than 500,000 leucocytes was 23,600 bacteria per c.c., while the average for milks containing more than 500,000 leucocytes was 231,350 per c.c. The average bacterial count for milk containing more than one million leucocytes was 216,636. The last milk in figure I which is milk No. 1 in Table VIII was omitted from these computations as this milk appeared very definitely abnormal.

THE BACTERIAL CONTENTS OF MILK AND THE METHYLENE BLUE REDUCTION TIMES

A considerable amount of work has been reported on the relationship of the plate counts to the methylene blue reduction times of milk. The literature shows that those reporting on this type of work were not able to find any close relationship between the results given by these two bacteriological methods. When we consider the fundamental principles involved in the two methods, we may expect no close relationship between the results given by these methods.

On the other hand, very little work has been reported on the relationship between the direct microscopic counts of bacteria in milk and the methylene blue reduction

times.

Troy (1925) reported on the comparison of the methylene blue reduction test and the direct microscopic counts in grading milk/in milk plants. This worker reported a correlation of 86.2 per cent with regard to their ability to place milk into the same grades. The correlation reported by this worker appears to be closer than might be expected for the class of milk he studied. However, similar results need not necessarily be expected in the study of udder milks.

In examining the bacterial count and the methylene blue reduction time curves in Figure I and also the data in Table VIII, we find that there is some relationship between the bacterial counts and the reduction times of these milks. There is a distinct rise in the reduction time curve with the rise of the bacterial count curve. A greater proportion of the milks with standard reduction times of less than 10:00 are to be found towards the high end of the bacterial count curve. There is, however, one significant thing that should be noted with regard to the curves in Figure I: this is the occurrence of milks with short reduction times and relatively low bacterial and leucocyte contents. Milks also appear which show the opposite results, namely, high bacterial counts and fairly long reduction times. The latter condition was probably due to slow rate of bacterial multiplication during the period when the tests were incubated in the water bath.

In cases where large numbers of leucocytes are found, one may attempt to attribute the short reduction times to the action of leucocytes, but where leucocytes are found in relatively low numbers such an explanation does not seem reasonable. Results of the above nature seem to indicate that there may be some factors other than bacteria and leucocytes which may be involved in the reduction of methylene blue in milk, primarily where there are insufficient bacteria to account for the reduction.

THE LEUCOCYTE CONTENTS AND THE BACTERIAL PLATE COUNTS OF MILK

On examining the data in Table IX, we find that the majority of milks with relatively high plate counts also contain large numbers of leucocytes. There are, however, a few very apparent exceptions to this rule. The most striking are the following: milks Nos. 3, 8, 18, 19 and 21. Each of these milks contained more than 2,000,000 leucocytes and each gave a plate count of less than 1,000. Referring to Table VIII, we find that large groups of bacteria were found in milks Nos. 3 and 8 when subjected to the extensive microscopic examination. This may also have been true of the other three milks, but these were not examined similarily. It should be noted that of the 92 milks each containing more

than 500,000 leucocytes, plate counts are reported for only 65. The average plate count of these 65 milks was 20,448. Milk No. 1, which gave a plate count of 7,875,000 was excluded from this average. The average plate count for milks containing 500,000 or less leucocytes per c.c. was 1,041. These averages are probably significant in spite of the fact that the plate counts varied from 5 to 511,000 for the former class of milks and from 25 to 17,500 for the latter class. We may therefore conclude on the basis of these averages that the plate counts are generally much higher for milks that are high in leucocyte contents than for milks containing relatively low numbers of leucocytes.

THE PLATE COUNTS AND THE METHYLENE BLUE REDUCTION TIMES OF UDDER MILK

The bacterial plate counts and the methylene blue reduction times of herd milks have been discussed in the first section of this paper, and a conclusion was reached, that no close relationship exists between plate counts and reduction times of such milks. Data in Table IX deal with a number of udder milks. These data show that there is a general relationship between the plate counts and the reduction times of these milks. Many of the milks showing high plate counts also show short reduction times. There

are, however, a large number of striking exceptions of which the following are the most outstanding, namely, milks Nos. 3, 5, 66 and 81. All these milks gave short reduction times and showed low plate counts. Referring to Table VIII, we find that only small clumps of bacteria were found in milks Nos. 3 and 66, while no chains or clumps were observed in milks Nos. 5 and 81. It should be noted that these results differ from those reported in Table III and discussed in the first section of this paper. The results reported in Table III show that large groups of bacteria were present in all of the herd milks which gave short reduction times and low plate counts.

Data in Table IX also show a number of milks with relatively high plate counts and long reduction times. The following seem to be good examples of such milks: milks Nos. 22, 59, 114, 123 and 142.

On the basis of these data we may conclude that there is only a general relationship between the plate counts and the methylene blue reduction times, but, there are also milks which show a negative relationship. An explanation for this negative relationship is likely seated in a combination of factors which tend to influence both of these bacteriological tests. Some of these factors are known to us at the present time, but there may be others which have not yet come to light. It is possible that there may be certain unknown factors that may affect the reduction time, particularly in the case of milks that come from infected udders.

THE LEUCOCYTE COUNTS AND THE METHYLENE BLUE REDUCTION TIMES OF MILK

The behavior of leucocytes with respect to the methylene blue reduction test of milk has not been studied as extensively as some of the other factors which influence this test. The reason that this has not been thoroughly investigated is likely due to the fact that many workers in the past were not inclined to attribute much importance to leucocytes and their action on the reduction time in practical milk control work.

Skar (1913) seems to have been the first to study this subject and as a result of his studies he concluded that leucocytes in milk have the power of reducing methylene blue.

Barthel (1917) found that milk from a single animal can become decolorized in say 1:00 because of high leucocyte content in the secretion from one or more of the glands. He was not, however, inclined to attribute to their action a great importance in practical milk control.

Skar (1931) again brought attention to the importance of leucocytes in the reduction test. Here he mentions the prevalence of infected herds in Norway where short reduction times of milk from individual animals are obtainable, but in these same areas the stable inspectors very seldom report quick reduction of the mixed milk from such herds, because of udder contamination. This worker states

that leucocytes in the numbers of roughly 6,700,000 per c.c. can not reduce the dye in milk if the test tubes stand still, but will reduce when the test tubes are turned over periodically, so that creaming is prevented. He attributes this condition to the fact that leucocytes go into the cream layer before the milk has decolorized. Creaming is, therefore, prevented by a periodic shaking of the samples during incubation, and thus allowing the leucocytes to decolorize the dye.

While the literature presents some fairly conclusive evidence that living cells including leucocytes have a reducing power, yet proof as to what extent the leucocytes found in milk reduce the dye awaits further investigation. In the discussion that will follow, an attempt will bemade to bring out experimental evidence which may seem to support the contentions of those who have concluded that leucocytes in milk have a reducing power. However, very important evidence will be presented which will show that leucocytes are likely not causing the reduction themselves, but that there may be certain other substances associated primarily with abnormal milks which actually cause reduction of the methylene blue.

An examination of data reported in Table IX shows a few significant conditions. The first of these is a rather close relationship between the leucocyte counts and both the standard and modified reduction times. This relationship is

also shown by the graph in Figure I. In the majority of instances, leucocyte count and reduction time curves rise and fall simultaneously. This, therefore, seems to show that there may be a closer relationship between leucocyte counts and methylene blue reduction times, than between bacterial counts (microscopic) and reduction times. A further analysis of the data in Table VIII shows that of the 64 milks containing leucocytes in excess of 500,000 per c.c., 38 (59.37 per cent) gave standard reduction times of less than 10:00; while of the 31 milks, each containing less than 500,000 leucocytes per c.c., only 2 (6.45 per cent) reduced the dye in less than 10:00. It should also be noted that of the 40 milks where chains or clumps of bacteria and leucocytes in excess of 500,000 per c.c. were found, only 9 (22.5 per cent) did not reduce the dye in less than 10:00. In only one of the two milks containing less than 500,000 leucocytes per c.c., and giving reduction times of less than 10:00, were large groups of bacteria observed.

Data in Table IX show that of the 57 milks which contained leucocytes in excess of 1,000,000 per c.c., 35 (61.4 per cent) gave standard reduction times of less than 10:00. If we group these milks by using 500,000 leucocytes per c.c., as a dividing line between the two groups, then we find that of the 92 milks containing leucocytes in excess of 500,000 per c.c., 43 (46.7 per cent) were decolorized in less than 10:00. While of the 71 milks containing 500,000 or less leucocytes per c.c., 2 (2.8 per cent) gave reduction

times of less than 10:00. The 2 milks referred to are milks Nos. 136 and 150. Milk No. 136 showed a leucocyte count of 180,000 and a standard reduction time of 6:45. This milk showed a bacterial count of 6,000 when 1,000 fields per smear were examined microscopically. These results, therefore, show clearly that there were insufficient bacteria and leucocytes present to account for the short reduction time. In the case of milk No. 150, the reduction time of 9:30 seems to be accounted for by the bacterial count of 43,200 when 1,000 fields per smear were searched. Table VIII seems to show that milks Nos. 48, 52, 65, 73, 81, 82, 136 and 158 all contained insufficient bacteria and leucocytes to account for their reduction times. All these cases show evidence of the existence in milk of some substance other than leucocytes and bacteria which was capable of reducing the dye in the reduction test.

It will be noted that there is a close relationship between the standard and modified reduction times. This relationship seems to be maintained with milks that are high in leucocyte contents and also with milks containing relatively low numbers of leucocytes. The following are some of the exceptions to the above rule: in the case of milks Nos. 26 and 66, the standard reduction times were considerably shorter than the modified times. Such a condition may be expected in case of short time reducing milks where the introduction of extra oxygen a s a result of periodic

shaking of the tubes is a greater factor than the prevention of the rise of bacteria with creaming. Conditions of the above nature are uncommon with long reducing normal milks. When such results are obtained with short reducing milks, the difference between the two reduction times has been found to be in no case as great as it is reported for the two milks mentioned above. The other exceptions are milks Nos. 60 and 68. With these milks the modified reduction times were short while the standard times were relatively long. This condition seems to support Skar's (1931) statement with regard to milks high in leucocyte content. However, both of these milks contained less than 1,000,000 leucocytes per c.c., therefore leucocytes apparently did not account for the reductions. The explanation for these differences between standard and modified reduction times seems to centre around the bacterial contents of these milks. Referring to Table VIII we find that large groups of bacteria were found in both of these milks. Both milks showed high microscopic counts of bacteria and considerably lower plate counts. These rather great differences between the plate counts and the microscopic counts confirm the findings of large groups of bacteria. Therefore, it seems that these differences between standard and modified reduction times were due entirely to the sweeping up of bacterial groups (Thornton (1930)) in creaming and thus preventing uniform reduction of the dye in the unshaken tubes.

Since these data show a fairly close relationship between standard and modified reduction times, and since the differences between these times appear small with short time reducing milks and greater with long time reducing milks, it appears as though the sweeping up of leucocytes with the cream in the standard technique had no bearing upon the reduction times of these milks. This conclusion is based on the fact that the correlation between standard and modified reduction times was found to be fairly close in spite of the great diversity between the leucocyte contents of the milks studied. Therefore, these data show different results from those reported by Skar (1931). Skar's conclusion that leucocytes in numbers of about 6,700,000 per c.c. are not able to reduce the dye when the tubes are not shaken does not seem to hold here. It may be stated that Skar's conclusion seems logical if the leucocytes have reducing properties. If this is the case there is no reason that the creaming effect should not have the same influence upon leucocytes as it has with respect to bacteria. The sweeping of leucocytes and bacteria in the standard methylene blue reduction test has been fairly well proven by many workers who have studied this problem, therefore in considering our data we may safely accept the fact that leucocytes are swept up with the cream in the unshaken tubes. If we accept this to be the case while analysing our data and find no marked difference between

standard and modified reduction times of milks relatively high in leucocyte contents, we may reasonably conclude that leucocytes in milk appear to have no power of reducing the dye in the reduction test.

Another point which is worth noting with respect to these data is the significant fact that milks Nos.18 19, 20, 21, 22, 24 and 27 each contained leucocytes in excess of 2 million per c.c. and none of these milks reduced the dye in less than 12:00 in the standard technique. Contrasting these milks with milks Nos. 65, 66, 73, 81 and 136, which in each case harboured less than 900,000 leucocytes per c.c. and reduced the dye in less than 7:00, we find additional evidence here that leucocytes in milk play no part in the reduction of methylene blue.

LEUCOCYTE COUNTS AND THE INITIAL BACTERIAL COUNTS CORRELATED WITH THE BACTERIAL COUNTS OF MILK AT THE MOMENT OF REDUCTION OF THE METHYLENE BLUE

Very little work has been reported on the bacterial content of milk at the moment of reduction of the methylene blue. Thornton and Hastings (1929) produced some important data on this subject. They used the plate method in making the determinations and reported an average plate count of

approximately 21 million and a variation of from 3.5 million to 45 million per c.c. of milk at the moment of reduction.

Skar (1913) reported data on 4 samples of milk, 2 of which contained 3,009,000 and 6,706,000 leucocytes per c.c. respectively. The first sample he reports had a reduction time of less than 12:00 and the second decolorized the dye in 8:00 to 9:00. Skar reports that both of these samples were examined microscopically after 23:00 standing in the thermostat and very few bacteria were found in the first and almost no bacteria appeared in the second sample after this incubation period. On the basis of these results, this worker attributed the reduction in each of these milks to the action of leucocytes.

While Skar's work appears quite significant, it may not have been extensive enough to justify his conclusions as the results reported were on the analysis of milk from only one cow. However, he reported that his conclusions were based on experiments of similar nature on other milks not included in his data.

The work undertaken in this study was along somewhat similar lines to that reported by Skar, except that a larger number of milks was studied and an extensive microscopic method was employed in the examination of milk smears. The milks that were chosen for this study contained varying numbers of leucocytes. These milks were subjected to thorough microscopic examinations immediately

after they were drawn from the udders, and also upon reduction of the dye. The reason for these thorough microscopic examinations was to find out whether these milks contained more bacteria than the plate method and the ordinary (60 fields per smear) microscopic examination seemed to show. It is believed that a microscopic examination of 1,000 to 2,000 fields per smear is more accurate than an examination of say 10 to 60 fields per smear. In analysing Skar's data, it was thought that more bacteria may have been present than he observed. He may have failed to observe these when examining only a few fields per smear.

An examination of data in Table X shows some striking results. We note a number of milks which were high in leucocyte contents and had short reduction times and also contained relatively few bacteria at the moment of reduction. These data seem to show that there were insufficent bacteria in milks Nos. 3, 4, 5, 7, 8, 10, 12, 13, 16, 26, 66 and 81 to account for the reduction of the dye in the times reported for these milks. By checking the leucocyte contents of these milks, one may be lead to conclude that leucocytes must have caused these short reduction times. However, two of these milks, namely Nos. 81 and 66 contained relatively few leucocytes as compared with the leucocyte contents of the other 10 milks. Here it appears that there were insufficient leucocytes (if leucocytes have a reducing power) and bacteria to account for the short reduction times. Both of these milks must be

considered as abnormal, since both were obtained from cows two days after parturition. On the basis of high leucocyte counts, short methylene blue reduction times, and the history of these milks, we may class all of the 12 milks as abnormal. Therefore, since all of these abnormal milks gave short standard reduction times, and all contained insufficient bacteria to account for the short reduction times, and since at least two of these milks were relatively low in leucocyte content, it appears that there were factors other than the leucocytes and bacteria which caused the short reduction times of the 12 milks, and that leucocytes played little or no part in the reduction of the dye.

It seems reasonable to suppose that there may be some unknown reducing substance in milk, which probably occurs in an increasing concentration in milks which show greater tendencies towards abnormality. This field, however, required further investigation before we may definitely ascribe the short reduction times to any unknown substance in the types of milks reported in this study. The results of this study lead us to the conslusion that leucocytes in milk are not as important in causing reduction of the dye in the reduction test as has been supposed by some of the investigators. Furthermore, these data seem to indicate that milks obtained from abnormal udders reduce the dye in a short time, irrespective of their leucocyte or bacterial contents. This, therefore, leads one to believe that certain milks appear to contain a reducing substance (or

substances) which probably occurs in an increasing concentration in milks exhibiting higher degrees of abnormality.

A rather unusual condition will be observed in connection with the reduction times of milks Nos. 26 and 66. In both cases the modified reduction times were of longer duration than the standard times. It appears that the substances which caused reduction in the unshaken tubes was not able to reduce the dye in the shaken tubes. However, partial reductions in the shaken tubes were observed prior to each shaking but upon shaking the tubes every half hour these partial reductions would disappear. This seems to show that the reducing substance must have had the power of reducing the dye but this power was not sufficient to cause complete reduction of the methylene blue during the course of the half hour period while the tubes remained at rest. We may now ask what caused the reduction in the respective times reported for the shaken tubes of these two milks. The answer is quite obvious that these reductions were caused by the bacterial activity, as the bacterial counts at the moment of reduction were 64,200,000 for milk No. 26 and 127,200,000 for milk No. 66. These results, therefore, show that bacteria were responsible for the modified reduction times but some other factor or factors were responsible for the reductions in the unshaken tubes.

It is also evident that neither leucocytes nor bacteria were responsible for reduction of the dye in the

standard tubes of milks Nos. 66 and 81, as the leucocyte contents were relatively low in both of these milks and insufficient bacteria to account for these reductions were observed in the smears made at the moment of reduction of the dye.

We should note that milks Nos. 27, 35, 39, 45, 50, 53, 55, and 56 each contained more than 1,000,000 leucocytes and none reduced the methylene blue in less than 9:00 in the standard technique. In the case of all these milks, there were enough bacteria found at the moment of reduction to account for their reduction times. This, therefore seems to furnish conclusive evidence that leucocytes did not cause reduction of the dye in these particular milks. It appears that these milks while relatively high in leucocyte contents were possibly less potent in the reducing substance which is believed to occur in many abnormal milks. It is probable that these particular milks were normal in all respects except that the leucocyte contents were relatively high. As the result of these conditions, it seems that reduction in these milks was not possible unless accounted for by the bacterial activity.

When we exclude those milks which appear to have contained insufficient bacteria to account for the reduction of the dye and only consider the milks which contained enough bacteria to account for the decolorization, we find the bacterial counts at the moment of reduction to be fairly

uniform. While these counts varied from 67,800,000 to 285,600,000, nevertheless they seem to be as uniform as may be expected. After all these are really estimates rather than actual counts. The average bacterial count at the moment of reduction for these 34 milks was 134,723,529, which is considerably higher than the average plate count of milk at the moment of reduction obtained by Thornton and Hastings (1929). The average bacterial count for the 12 milks which contained insufficient bacteria to account for their reduction times was 228,808.

In connection with milks Nos. 3, 5, 7, 8, 10 and 13, a decrease in the numbers of bacteria at the moment of reduction was noted. In these 6 milks there were actually fewer bacteria found in the smears made at the moment of reduction than in the smears made before the milks were placed in the water bath. These decreases in the bacterial counts as reported here may have been due to the inaccuracy of the method of counting or to the phagocytic action of leucocytes upon the bacteria.

Incubation was continued in case of milks Nos. 3, 5, 7, 8, 13, 26, 66, and 81 and results are shown in Table XI. All of these milks were incubated in the water bath for a period of 8:00 including the time before the milks were reduced. In each case a smear was made at the moment of reduction and then the test tube was again returned to the bath where it remained until a total incubation period of 8:00 was reached. When this period was reached

another smear was prepared from each milk, therefore the counts reported in Table XI are representative of the smears made after the 8:00 incubation period.

An examination of the data in Table XI shows that only one milk actually contained large numbers of bacteria: however, this same milk did not show enough bacteria to account for its reduction time of 0:55 (Table X).

Comparing the initial bacterial count, the bacterial count at the moment of reduction (Table X) and the bacterial count after the prolonged incubation (Table XI) for milk No. 13, we note that there is very little difference between these counts. In fact the count obtained after the extended incubation period was numerically lower than the initial bacterial count of this milk. This, therefore, seems conclusive evidence that there were factors which tended to prevent bacterial multiplication or which destroyed the bacteria present in these milks under the conditions of the methylene blue reduction test.

The plate counts of milks Nos. 8, 66, and 81 are reported in Table XI. These milks were plated after the 8:00 incubation period, i.e., during the same period when Breed smears were prepared. These plate counts are in all cases lower than the corresponding microscopic counts. They also show that there were insufficient bacteria present to account for the recorded reduction times. Comparing these plate counts with the initial plate counts reported in Table X, we find a great increase in the numbers of bacteria during

the incubation period, while the corresponding microscopic counts of these milks do not show any such high percentage of increase during the same period. This may possibly be explained by the fact that while shaking the tubes large groups of bacteria were broken up and clumping may have been prevented.

These figures serve as further evidence that there were insufficient bacteria present in these 8 milks to account for the respective reduction times.

It was observed that certain decolorized milks when the tubes were shaken and again replaced in the water bath did not decolorize again within a few minutes as would be expected, and as is the case with milks which are reduced due to bacterial activity. These observations have been made in connection with milks Nos. 8, 66 and 81. These milks were thoroughly shaken after the 8:00 incubation in the bath and the original blue color reappeared. The test tubes were again returned to the bath and observations for reduction were made every half hour. The tubes received no further agitation. Milk No. 8 was reduced in 2:15 (this is comparable with the first reduction time of 2:10), while milks Nos. 66 and 81 were not decolorized when they were last examined 3:00 after the tubes were shaken.

In view of these results, it appears that there is justification for a further investigation of this problem. This seems necessary before we can explain the results mentioned above.

BOVINE AND RABBIT BLOOD LEUCOCYTES ADDED TO MILK AND THEIR EFFECT ON THE METHYLENE BLUE REDUCTION TIME OF MILK

Data which we have so far presented dealt only with the role of the milk borne leucocytes in the reduction of methylene blue in milk. The results of this study have lead the author to the conclusion that leucocytes found in milk appear to have no effect on the reduction of methylene blue in milk. However, before definitely concluding that leucocytes have no reducing properties in milk, it seemed desirable to study the effect of adding Bovine and Rabbit blood leucocytes to milk.

Since a search through the literature did not uncover any work on the action of blood borne leucocytes in milk on the reduction of methylene blue, it is therefore impossible to present any review on this phase of the subject. However, Gay and Oram (1933) deal with the action of Rabbit, Dog and Guinea Pig leucocytes on methylene blue in broth. These workers studied the effect on leucocytes of a substance named Streptococcus leucocidin, and found that this substance caused the disintegration of leucocytes, and as aresult of this the leucocytes lost their reducing properties. St. leucocidin is produced as a metabolic product of certain bacteria, notably of the Streptococcus and Staphylococcus types. This product is claimed to exercise a fatal effect on the protective leucocytes not by repelling them but by destroy-

ing them after the micro organisms have become incorporated in the leucocyte. These workers used methylene blue as an indicator of living cells. The data submitted in these investigations show clearly that leucocytes suspended in broth are definitely capable of reducing methylene blue. The table below reproduced from Gay and Oram (1933) illustrates the above contention very clearly:

Reducing Power of Living and Dead Leucocytes and effect of Leucocidin

No.	Description	Reduction in two hours
1.	Streptococcus filtrate 2 c.c. + Leucocytes 0.3 c.c. + Methylene Blue 2 drops 0.1 p.c. solution	0
2.	Broth 2 c.c. + Leucocytes o.3 c.c. + M.B. 2 drops	+ + + +
3.	Broth 2 c.c. + Leucocytes heated at 56°C. for 1 hour 0.3 c.c. + M.B. 2 drops	0
4.	Streptococcus filtrate 2 c.c. + M. B. 2 drops	0
5.	Broth 2 c.c. + M.B. 2 drops	0

This exudate contained 50,000 Leucocytes per cubic millimenter of which 91 per cent were polymorphonuclear.

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Blood was drawn aseptically from the External Jugular vein of a Jersey cow into a flask containing sterile physiologic saline solution. The leucocytes were separated

by repeated centrifugalizations, decantations and washings with sterile saline until finally a clear suspension of approximately 500 million cells per c.c. was obtained. This leucocyte suspension was added to fresh udder milk according to the proportions outlined in Table XII.

The Rabbit blood leucocytes were obtained from the peritoneal cavity by introducing physiologic saline solution therein. About 200 c.c. of saline were introduced into the peritoneal cavity early in the morning and 100 c.c. late in the afternoon; three hours later about 200 c.c. of exudate were obtained by aspiration. By centrifugalizing, decanting and adding fresh saline, a smaller volume was obtained which contained approximately 10 million leucocytes per c.c.. This suspension was immediately added to fresh udder milk according to the proportions outlined in Table XIII.

A careful analysis of data reported in Tables XII and XIII shows quite conclusively that the leucocytes which were added to these samples of milk exhibited no measurable effect on the methylene blue reduction times of these milks. There may have been a possibility that these leucocytes lost their reducing power immediately after they were introduced into the milk. This seems to be borne out by the fact that these cells appeared to be in a disintegrated condition in the smears that were prepared from the milk samples immediately following the addition of the leucocyte suspension to the milk. The majority of the cells appeared as skeletons

and did not stain properly. This condition was more pronounced in connection with Bovine leucocytes than was the case with respect to Rabbit leucocytes. It is rather difficult to explain these disintegrations when the leucocytes were introduced into the milk; such disintegration was, however, not observed in the smears that were made from the leucocyte suspensions prior to their addition to milk.

Data reported in Tables XII and XIII show clearly that the addition of varying numbers of blood leucocytes to samples of milk caused no measurable change in the reduction times of these milks. The microscopic examinations of smears made at the moment of reduction serve as further proof that leucocytes played no part in the reductions of methylene blue in these milks. In every smear examined microscopically enough bacteria were present at the moment of reduction to account for the respective reduction times.

On the basis of these data, we may conclude that both Bovine and Rabbit blood leucocytes when introduced into aseptically drawn milk have no measurable effect on the reduction of methylene blue in milk. Incidentally, this study, therefore, further confirms the conclusions made with respect to the role of milk borne leucocytes in the reduction of methylene blue in milk.

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P A R T III

THE INCIDENCE OF MASTITIS

AMONG THE COWS IN THE EDMONTON MILK SHED

INTRODUCTION

In connection with the studies reported in the first two parts of this thesis, data are available which permit some comment on the incidence of mastitis among the cows in this district.

Within the last few years, a great deal of work has been reported on mastitis among the cows in various parts of the continent. Valuable data have been collected on all phases of this malady. Some of the most outstanding work has been reported by the following reaearch workers: Hucker, et. al. (1932), Hucker (1933, 1933), Hucker and Lee (1932), Rosell (1933) Hucker and Udall (1933) and Cherrington et. al (1933). The reader is referred to these papers for information on this subject which can not be given here.

Most of the workers have definitely associated the excessive leucocyte content of milk with infections of the udder. There still is, however, a great deal of controversy among the different workers as to what leucocyte content of milk should be taken as a dividing line between normal and abnormal milk.

Hucker (1933) states that cows producing milk containing more than 150,000 leucocytes per c.c. show past or present histories of mastitis. This worker further states that milk with a leucocyte count of 500,000 almost invariably comes from an infected udder.

Cherrington, et. al. (1933) state that milks with

leucocyte contents of over 100,000 per c.c. almost invariably come from abnormal udders.

In reviewing the contentions of the different workers, one appreciates that it is a difficult task to set a leucocyte standard for normal milk. However, since most of the authorities are in agreement that a leucocyte content of 500,000 per c.c. in milk is indicative of abnormality of the udder, it seems safe to take this figure as an arbitrary line of demarcation between normal and abnormal milk. Therefore, in the discussions in this paper, cows producing milk with leucocyte content of over 500,000 per c.c. will be considered abnormal or suffering from mastitis.

THE LEUCOCYTE COUNTS OF MILK DRAWN FROM 163 UDDERS

The leucocytes from the standpoint of their quantitative content in milk have been studied rather extensively. The literature reveals that there are great variations in leucocyte counts of milk from apparently normal udders.

Breed (1914) discussed leucocyte counts of milk from 122 apparently normal individual cows. He found an average leucocyte content of 868,000 per c.c.. Of the 122 cows, only 59 gave milk with leucocyte content of less than 500,000 per c.c.

Copeland and Olsen (1926) obtained an average leucocyte count of 657,000 for 40 cows.

Chemington, et. al. (1933) reported leucocyte determinations of milk from 6 normal and 7 abnormal cows. These workers reported 144 representative counts of milk from the normal cows, and their data show that 94 per cent of these samples contained more than 10,000 leucocytes per c.c.; 11 per cent contained more than 50,000, and 3 per cent contained more than 100,000 leucocytes per c.c.. Of the 168 counts pertaining to the 7 diseased cows, 99 per cent showed more than 50,000 leucocytes per c.c., 96 per cent more than 100,000, and 40 per cent contained more than 1,000,000 leucocytes per c.c.. These workers conclude that milk from normal udders usually contains less than

50,000 leucocytes per c.c., whereas milk from infected udders almost invariably contains more than 100,000 leucocytes per c.c.

The data presented in Table IX give some idea of the extent of leucocytes in milk produced by the cows in this district.

Milks drawn from 163 cows representative of 14 herds were studied. Of these 163 milks, 156 were apparently normal, while the remaining 7 milks were classed as abnormal. Among these 7 milks, milks Nos. 66 and 81 were classed abnormal because they were drawn from cows 2 days after parturition, and the remaining 5 milks were classed abnormal due to their yellowish brown appearance and stringy consistency. In this study, the milks that were shipped to the distributing plants were considered as apparently normal, while the milks that were not sold on account of their physical appearance were considered abnormal.

Table IX presents data showing the leucocyte contents of 163 milks. Excluding the 7 abnormal milks, namely milks Nos. 1, 2, 3, 4, 7, 66 and 81, we have left for our consideration the 156 apparently normal milks. Upon examing the data pertaining to the normal milks, we find that 86 (55.1 per cent) of the cows produced milk containing 500,000 or more leucocytes per c.c.. It will be noted that only 10 (6.4 per cent) of the cows produced milk with leucocyte content of less than 100,000 per c.c.. The average leucocyte content for the 156 milks was 1, 187,000 per

c.c., which seems higher than the averages reported by other workers. The highest leucocyte count obtained in apparently normal milk was 15,960,000, and the lowest was 30,000. The milk with the leucocyte count of 15,960,000 gave a plate count of 1,000 and a microscopic count of 48,000 bacteria per c.c. when 2,000 fields per smear were examined. No longchain streptococci were observed in this milk during the course of the microscopic examination. The standard methylene blue reduction time for this milk was 1:45. It is therefore apparent that the short reduction time and the high leucocyte count appear to indicate that this milk was abnormal. This condition, however, was not shown by the plate count or by the microscopic examination for bacteria.

The highest leucocyte count obtained for the abnormal milk was 120,000,000. The average for the 5 abnormal milks was 50,320,000 leucocytes per c.c.: milks Nos. 66 and 81 were excluded from this average.

If one were to apply the arbitrary leucocyte standard of 500,000 leucocytes per c.c. as indicative of pathological conditions in the udder, then 55.1 per cent of the 156 normal cows included in this study would fall into the abnormal class. On the other hand, if a leucocyte count of 100,000 was adopted in this classification, then 93.6 per cent of these cows would have to be classed as abnormal or suspicious.

It is evident from these data and from the work reported by other investigators that in order to set a definite leucocyte standard for normal milk, more research is required on the significance of leucocytes in milk, and on their relationship to specific udder infections.

THE INCIDENCE OF MASTITIS IN ONE HERD OF 27 COWS AS SHOWN BY THE LEUCOCYTE CONTENT OF QUARTER MILKS

Since a leucocyte count of 500,000 has been adopted as a dividing line between normal and abnoral milk, it was thought desirable that the variations in the leucocyte counts of milk from the different quarters of the same udder, and of milk drawn at different times during the lactation period should be studied. This study was also undertaken with anticipation of securing some information on the incidence of mastitis among the cows of a single herd, based on the examination of quarter milks.

Copeland and Olsen (1926) reported leucocyte counts in milk from individual quarters of 40 cows and their data show great variations in leucocyte counts of milk from the different quarters of the same udder.

Hucker, et. al. (1932) reported individual quarter leucocyte counts of 221 cows which had been condemned by the tuberculin test. Their data also show great variations.

Baker and Breed (1920) presented data on leucocytes and epithelial cell counts of individual quarters of 21 cows. Their results also show high variations in both leucocyte and epithelial cell counts of the different quarter samples from the same udder.

The work reported on the numbers of leucocytes in milk drawn at different times during the same period of lactation also show that the number of leucocytes secreted into the milk vary considerably at different periods.

Ross (1911) studied the leucocyte contents of milk from 3 cows covering a period of 5 to 7 months and found in the case of 1 cow a variation of from 21,000 to 272,000 leucocytes per c.c. and with another cow which gave a high count, the leucocytes varied from 107,000 to 1,060,000. This worker concludes that the numbers of leucocytes in milk from individual cows vary mostly within certain unstated limits.

Cherrington, et. al. (1933) presented data on the leucocyte content of milk drawn at 6 different periods from 6 normal and 7 abnormal cows. Their data also show wide variations.

Breed (1914) studied the relationship between leucocyte counts and the period of lactation, and on the basis of the results obtained from 246 tests, he draws the following conclusion:

> "It seems clear that the cell counts of the first 3 days of lactation averagemuch higher than those of later periods, although usually high

> cell counts are seen in almost all of the later periods. When the counts are separated into 2 groups at the 500,000 per c.c. mark, it is found that the ratio between the number of counts under this mark to the number of counts over this mark during the first 6 months of lactation (exclusive of counts obtained from colostrum milk) is 100:52. The similar ratio, for the counts obtained during the second 6 months is 100:82. That is the majority of the high cell counts here given occurred during the latter part of the lactation period."

Data presented in Table XIV give leucocyte counts of individual quarter milks from 27 cows. The samples were taken at 3 different periods during the evening milkings. All these cows were in the same herd, and were all considered normal by the owner. The time elapsing between the first and second sampling periods was 1 day, and between the second and third sampling periods, 3 days elapsed.

An examination of these data reveals that there are great variations between the leucocyte counts of milk from the different quarters of the same udder. These variations are of the same nature as those reported by other investigators. The wide variations in the leucocyte counts of milk from the different quarters of the same udder are shown by the figures pertaining to the following cows: Nos. 1, 2, 4, 7, 8, 11, 15, 16, 18, 19, 22, 25, 26, and 27. Milk from cow No. 25 gave results that are the most outstanding in this respect.

The figures for the 3 different milking periods also show wide variations in spite of the fact that all of the samples were drawn within a period of less than 1 week.

The following milks are worth noting with regard to the wide variations: namely R.F. milk from cow No. 1; R.F. No. 8; L.F. No. 15; L.F. No. 18; R.R. and L.R. No. 25; L.F. and R.R. No. 26 and L.F. No. 27.

Of the 312 quarter milks reported in Table XIV, 120 (38.4 per cent) contained more than 500,000 leucocytes per c.c., and 50 (15.6 per cent) contained less than 100,000 leucocytes per c.c.. Therefore, if we set a leucocyte count of 500,000 as a dividing line between normal and abnormal milk, then 120 (38.4 per cent) of these quarter milks would be classed as abnormal.

On the other hand, if we apply the leucocyte count of 500,000 as a line of demarcation between normal and abnormal milk to the 27 cows, we find that 23 (85.1 per cent) of these cows would be classed as producers of abnormal milk from 1 or all quarters during 1 or all of the 3 sampling periods.

This analysis, while only arbitrary, seems to show that mastitis is undoubtedly a very important economic factor in this herd. On the basis of the arbitrary standard adopted in this study, we may conclude that the evidence of mastitis among the cows in this herd appears to be much higher than would generally be anticipated.

THE INCIDENCE OF MASTITIS AMONG THE COWS IN 13 HERDS

Data in Table XV are representative of 149 cows from 13 herds in this district. The figures representing the number of cows within the herd do not in all cases signify the total number of cows in the herd. In herds Nos. 3, 6, 12 and 13, all the milking cows were studied, while the cows reported on from the other 9 herds were selected at random and these may not necessarily be representative of the entire herd in each case. Another point which should be considered is the fact that the herds reported upon were selected from among those that were managed by herdsmen who were having some difficulty in producing milk that would stand up to the standard set by the City's Health Department. The standard referred to is based on the methylene blue reduction test. All milks giving standard reduction times of less than 5:30 are rejected. Work reported by Thornton, et. al. (1934) on the sources of milk contamination in this distrct shows that the difficulty in producing milk which would stand up to this standard did not centre in the herd itself but in the methods of production and handling of milk. The utensils were found to be the main source of milk contamination. There was, however, one possible exception, and this was in connection with herd No.6 (Table XV). During the survey on this farm, mixed herd milk

produced in bacteriologically sterile utensils gave a standard reduction time of 5:45. We note (Table XV) that a rather high percentage of the cows in this herd showed symptoms of infection. This particular herd was managed in a very unsatisfactory manner, and in this respect was not by any means representative of the average herd in this distrct.

Data reported in Table XV classify the milk from 149 cows representative of the 13 herds. An examination of these data shows that 80 (53.7 per cent) of the cows within the 13 herds produced milk containing more than 500,000 leucocytes per c.c. These data, therefore, show that 53.7 per cent of the cows among the 13 herds showed symptoms of mastitis when their milk was classified on the basis of the leucocyte standard adopted in this study. The results obtained are comparable with those reported on the incidence of mastitis among the herds in other parts of this continent. This disease undoubtedly is of a pronounced economic significance to the dairymen in the Edmonton district, and it seems that studies, of the best methods of its diagnosis and treatment, are well justified.

MASTITIS MILK IN RELATION TO THE BACTERIOLOGICAL QUALITY OF MIXED HERD MILK

Reference has already been made to Skar's (1931) work where he states that short reduction times of milk from individual animals are obtainable because of udder contamination, but on the otherhand it is very seldom that short reduction times of mixed milk from such herds are reported where the reduction is attributed to udder contamination.

The work by Thornton, et. al. (1934) shows that the prevalence of mastitis in the herd may but very rarely reveal itself in the reduction test of the mixed herd milk.

The work reported in Table XVI covers a study of 7 herds. The number of cows in each herd that show symptoms of mastitis are reported in percentage of the total number of milking cows within the respective herd. In segregating the cows showing symptoms of mastitis from the healthy ones, the same arbitrary standard was used here as is outlined in Table XV. In the case of each herd, the cows were milked in sterile utensils and in producer's own utensils which were in no case properly sterilized, but which received varying degrees of cleaning or scalding.

On examination of the data in Table XVI, we note a rather significant condition in connection with herd No. 2. Here we find a short reduction time of milk produced in sterile

utensils. The sterile utensil milk gave a standard reduction time of 5:45, while the milk produced in producer's utensils during the same milking reduced the dye in 8:45. Comparing the reduction times of the sterile utensil milk and the producer's utensil milk from herd No. 2 with the milks from the other 6 herds, we find that with the milks from these 6 herds the reduction times of sterile utensil milks were in all cases over 10:00. Also with every one of these 6 herds the reduction times of milks from producer's utensils were shorter than the reduction times of milks drawn during the same milking into sterile utensils. The results pertaining to herd No. 2 do not, therefore, compare with the results given by the milks from the other 6 herds. It seems that this difference may be explained as being due to the fact that most of the milk which was milked in sterile utensils in connection with herd No. 2 probably came from diseased udders, while the milk coming in contact with the producer's utensils during this milking may have come from the majority of the normal udders.

We note the highest incidence of mastitis (as obtained by the standard adopted here) in herd No. 1 where 66.6 per cent of the cows produced milk tending to be abnormal, and the lowest incidence of mastitis was shown in herd No. 4 where 37.5 per cent of the cows showed evidence of mastitis as revealed in the examination of their milk. Comparing the reduction times of sterile utensil milks from these 2 herds, we find no significant difference between

these reduction times. The fact should be noted that outside of herd No. 2 the reduction times of sterile utensil milks from all 6 herds were quite uniform in spite of the fact that the incidence of mastitis among the cows within the different herds varied considerably. It will be noted that high variations prevailed among the reduction times of milk produced in producer's utensils in connection with all 7 herds. These variations were no doubt due to the fact that the unsterilized utensils used on these 7 farms contributed varying amounts of contamination.

In view of these results we may conclude that the incidence of mastitis in the herd usually bears very little relationship to the methylene blue reduction time of mixed herd milk. There may be exceptions, however, where the high incidence of mastitis in the herd may itself cause short reduction time of mixed herd milk, but such occurrences are believed to be rare and only confined to herds under the most careless management. Data reported here, therefore, show that short reduction times of mixed herd milks may very seldom be attributed to diseased udders, but are primarily due to sources of milk contamination other than the cow's udder.

THE METHYLENE BLUE REDUCTION TEST AS A TEST FOR THE DETECTION OF MASTITIS AMONG THE COWS

Since a fair degree of relationship has been observed between the high leucocyte counts of milk and the incidence of mastitis, and also since a fairly close relationship has been noted between the leucocyte counts and the methylene blue reduction times, it was thought advisable to summarize the data collected during the course of this study and find to what extent the reduction test may be used in the detection of mastitis.

Throughout this discussion the cows producing milk with more than 500,000 leucocytes per c.c. will be considered abnormal or suffering from mastitis. The standard reduction times of these milks will be studied and an arbitrary standard reduction time of 10:00 or less will be taken as indicative of mastitis milk. An attempt will therefore be made to arrive at the value of the reduction test in detecting mastitis among the cows.

A summary of data reported in Tables IX, XIV, and XV shows that of the 624 milks, 292 (46.79 per cent) contained more than 500,000 leucocytes per c.c.. Of the 292 milks giving leucocyte counts higher than 500,000, 115 (39.38 per cent) reduced the dye in 10:00 or less in the standard technique. On the other hand, of the 332

milks containing less than 500,000 leucocyte per c.c., only 9 (2.71 per cent) gave standard reduction times of 10:00 or less. These data, therefore, show that the methylene blue reduction test is able to segregate approximately 40 per cent of the milks which are classified abnormal on the basis of the leucocyte count of 500,000.

If an assumption is made on the basis of the work reported by Hucker, et. al. (1932) and Hucker, (1933) that the leucocyte count in excess of 500,00 incriminates approximately 80 per cent of the cows showing symptoms of mastitis, then the reduction test may be expected to detect approximately 30 per cent of the infected cows.

On the basis of the data summarized above, it seems reasonable to conclude that the methylene blue reduction test has some value in the detection of abnormal milk. It seems that this test is able to detect the majority of milks which come from cows showing definite symptoms of mastitis. This is shown in Tables IX and XIV. When we examine the standard reduction times of milks containing more than 3 million leucocytes per c.c., we note that of the 28 of these milks, 24 (85.71 per cent) reduced the dye in less than 10:00.

DISCUSSION OF RESULTS

PART I

The results obtained on the accuracy of the available methods of measuring bacteria in herd milk, show that the methylene blue reduction test probably measures the bacterial populations in milk where bacteria exist in the form of large groups more accurately than does the plate method or the routine microscopic method. This contention is borne out by the data pertaining to milks which gave short reduction times and low plate counts, and which were found to contain enough bacteria to account for the short reduction times when as many as 1,000 fields per smear were examined microscopically, or where smears prepared at the moment of reduction showed sufficient bacteria present to account for that phenomenon. The discrepancies between plate counts and reduction times of herd milks are apparently due to the faultiness of the plate method when applied to milks that harbour bacteria in the form of large groups.

The inaccuracies of the direct microscopic method in estimating bacteria in milk seem to be due primarily to the uneven distribution of bacterial cells and groups of bacteria throughout the smear and also to the fact that it is not always possible to obtain a representative sample of milk for the smear with the 0.01 c.c. pipette.

The results show that the 1,000 field per smear microscopic bacterial counts are probably closer to the actual bacterial contents than the counts obtained when only 60 fields per smear are searched. This is in accordance with what may be expected when all the factors which tend to influence the routine direct microscopic method are taken into consideration.

The results on the accuracy of the direct microscopic method as it is applied to the estimation of the leucocyte content of milk show that this method does not necessarily give accurate results. In view of the fact that wide variations were obtained among the replicate counts from the same smear, and also among the counts made on replicate smears from the same milk, it appears that these variations were due to the uneven distribution of leucocytes throughout the smears. These results, therefore, seem to show that the 0.01 c.c. pipette probably discharges fairly uniform samples of milk for the smears.

PART II

The results pertaining to the studies on the role of leucocytes in the methylene blue reduction test show that leucocytes in milk probably do not exhibit any reducing properties. While it is true that a close relationship has been found between the reduction times and leucocyte counts,

many exceptions were also observed. The results seem to show that milks which come from abnormal udders reduce the dye in a short time irrespective of their leucocyte and bacterial contents. It, therefore, appears, that an unknown reducing substance is probably associated with the milks containing large numbers of leucocytes. This seems to indicate that the close relationship between leucocyte counts and reduction times is likely established indirectly through the relationships between the presence of some reducing substance in milk and the incidence of abnormality in the udders.

A summary of data pertaining to the role of milk borne leucocytes and Rabbit and Bovine blood leucocytes in the reduction test of milk, leads to the conclusion that leucocytes in milk (milk borne or added to milk) exhibit no measurable effect on the reduction of methylene blue.

The results pertaining to milks where insufficient leucocytes and bacteria were found to account for the reduction of the dye serve as further proof that there is probably present in such milks some unknown substance which is capable of reducing the dye in the reduction test.

PART III

A relatively high average leucocyte content of milks from apparently normal cows in this district seems to indicate that probably a high percentage of the cows in this district have had past or present histories of mastitis.

In view of the fact that the majority of the workers are in agreement that a leucocyte content of over 500,000 per c.c. is indicative of abnormality of the udder, it seems that it is not unreasonable to adopt this figure as an arbitrary line of demarcation between normal and abnormal milk.

A standard reduction time of over 10:00 for aseptically drawn udder milk seems to be reasonable to expect for normal milk. It appears that udder milks which reduce in less than 10:00 invariably come from abnormal or infected udders. The results of these studies, therefore, seem to indicate that the reduction test based on the standard reduction time of 10:00 or less is capable of segregating approximately 40 per cent of the milks classed abnormal on the basis of the leucocyte count in excess of 500,000.

SUMMARY AND CONCLUSIONS

The studies on the leucocytes and the reduction of methylene blue in milk lead to the following results and conclusions:

1. The results show that the plate counts often give misleading information on the bacteriological condition of herd milks where bacteria tend to occur in large aggregates.

2. It appears that the methylene blue reduction test measures the bacterial content of milk where bacteria tend to occur in large groups more accurately than does the plate method. This, therefore, indicates that the reduction test is probably not affected by clumping of bacteria to the same degree as is the plate count.

3. The bacterial content of milk at the moment of reduction is higher than is indicated by the plate count.

4. The microscopic count based on the examination of 60 fields per smear may be very misleading when applied to milks that harbour relatively few bacteria and those milks where large groups of bacteria are found.

5. The inaccuracies in the direct microscopic method of estimating bacteria in milk are mainly due to the uneven

distribution of singlecocci and of chains and clumps of bacteria throughout the stained smear.

6. The results show that the 0.01 c.c. pipette does not necessarily withdraw a bacteriologically representative sample of milk for the smear.

7. The average maximum deviation from the mean for the microscopic bacterial counts (based on the examination of entire smears) was 74.06 per cent, as contrasted with an average of 35.07 per cent for the leucocyte counts. In view of these results it may be concluded that the leucocyte counts are reasonably accurate for routine work.

8. A large proportion of the milks containing more than 500,000 leucocytes per c.c. were found to harbour clumps or chains of bacteria when 1,000 fields per smear were searched.

9. The average bacterial count of milks containing more than 500,000 leucocytes per c.c. has been found to be much higher than the average for milks containing less than half a million leucocytes.

10. Only a general relationship has been noted between microscopic bacterial counts (1,000 fields per smear) and reduction times of udder milks.

11. A fairly close relationship has been noted between reduction times and leucocyte counts of udder milks.

12. A number of short time reducing milks gave results which show that these milks contained insufficient bacteria and leucocytes to account for the reduction.

13. A close relationship has been observed between standard and modified reduction times of milks containing large numbers of leucocytes. This is indicative that the sweeping up of leucocytes with the cream in the standard tests had no bearing upon the reduction times. Therefore, such results seem to indicate that leucocytes in milk exhibit no reducing properties.

14. Abnormal milks seem to give short reduction times irrespective of their bacterial and leucocyte contents. Where enough bacteria are not found in such milks to account for the reduction, it appears that the reduction is due to the presence of some unknown substance which causes the reduction of the dye in such milks.

15. Bovine and Rabbit blood leucocytes when added to udder milk were found to exhibit no measurable effect on the methylene blue reduction time of such milk.

16. An average leucocyte count of 1,187,000 was obtained for 156 apparently normal milks. Of the 156 cows, 86 (55.1 per cent) produced milk containing more than 500,000 leucocytes per c.c.

17. High variations were obtained between the leucocyte

counts of milk from the different quarters of the same udder.

18. The results show wide variations between the leucocyte counts of milk drawn at different times during the lactation period.

19. A high incidence of mastitis among the 27 cows within one herd has been observed.

20. A study of the incidence of mastitis among the cows in 13 herds reveals that this disease is undoubtedly of an important economic significance to the dairymen in the Edmonton district.

21. It has been shown that short reduction times of mixed herd milks may but very seldom be attributed to diseased udders.

22. The methylene blue reduction test may be expected to detect approximately 30 per cent of milk coming from abnormal udders.

23. It has been found that 85.71 per cent of the milks containing more than 3 million leucocytes per c.c. reduced methylene blue in less than 10:00 in the standard technique.

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BIBLIOGRAPHY

BAKER, J. C., and BREED, R. S.
1920 The reaction of milk in relation to the presence of blood cells and specific bacterial infections of the udder. N. Y. (Geneva) State Agr. Exp. Sta., Tech. Bul. No. 80.

_____, and VAN SLYKE, L. L.
1919 A method for the preliminary detection of abnormal milk. N. Y. (Geneva) State Agr. Exp. Sta., Tech. Bul. No. 71.

BARTHEL, Chr.
1917 Weitere Untersuchungen über die Reduktaseprobe, sowei Vergleiche mit einigen anderen neueren Milch hygienischen Untersuchungsmethoden. Zeitschr. Untersuch. Nahr. u. Genussmittel, XXXIV, 137 - 164.

BREED, R. S.
1929 The sanitary significance of leucocytes in milk. N. Y. (Geneva) State Agr. Exp. Sta., Bul. No. 568.

_____
1914 Cells in milk derived from the udder. N. Y. (Geneva) State Agr. Exp. Sta., Bul. 380.

_____, and BREW, J. D.
1916 Counting bacteria by means of the microscope. N. Y. (Geneva) State Agr. Exp. Sta., Tech. Bul. No. 49.

_____, and STIDGER, I. R.
1911 The number of cellular elements in milk. Jour. of Inf. Dis., VIII, 361 - 385.

BREED, R. S., and STOCKING, W. A., Jr.
1920 The accuracy of bacterial counts from milk samples. N. Y. (Geneva) State Agr. Exp. Sta., Tech. Bul. No. 75.

CHERRINGTON, V. A., HANSEN, H. C., and HALVERSEN, W. V.
1933 The leucocyte content of milk correlated with bacterial count and hydrogen-ion concentration for the detection of mastitis. Jour. Dai. Sci. XVI, 59 - 67.

COPELAND, L. H., and OLSEN, T. M.
1926 The bacterial flora of normal cow's udder. So. Dakota Agr. Exp. Sta., Bul. No. 218.

DORNER, W.
1933 La durée de la decolcration a l'epreuve de la reductase est-elle fonction de la teneur bacterienne du lait? Le Lait XIII, 595 - 608.

GAY, F. P. and ORAM, FLORENCE
1933 Streptococcus Leucocidin Jour. of Immunology, XXV, 501 - 525.

HUCKER, G. J.
1933 The Laboratory detection of bovine mastitis. N. Y. (Geneva) State Agr. Exp. Sta., Bul. No. 626.

_____
1933 Relationship of leucocytes and streptococci to fibrosis of the udder. Amer. Jour. of Public Health, XXIII, 237 - 245.

_____, and LEE, DOROTHY, A.
1932 Mastitis: II. The use of certain dyes in the treatment of mastitis. N. Y. (Geneva) State Agr. Exp. Sta., Tech. Bul. No. 205.

_____, TRUDELL, F., and JENNINGS, W. S.
1932 Mastitis: I. The incidence and detection of sub-climical streptococcus mastitis. N. Y. (Geneva) State Agr. Exp. Sta., Tech. Bul. No. 199.

_____, and UDALL, D. H.
1933 Relation between the presence of fibrotic tissue in the udder and streptococci or cells in freshly drawn milk. Cornell Veterinarian, XXIII, 32 - 39.

JOHNS, C. K.
1930 A modification of the methylene blue reduction

test and its comparative value in estimating keeping quality of milk. Scientific Agriculture, XI, 171 - 190.

LIND, C.
1920 The value of methylene blue reduction test as compared with the plate method. The Milk Dealer, IX, 24 - 42.

MILES, C. S.
1933 Some observations on the methylene blue reduction test. The Jour. of the Ministry of Agr., XL, 414 - 419.

PENNINGTON, M. E., and ROBERTS, E. L.
1908 The significance of leucocytes and streptococci in the production of high grade milk. Jour. of Inf. Dis. V, 72 - 84.

PRESCOTT, S. C., and BREED, R. S.
1911 The determination of the number of body cells in milk by the direct method. Jour. of Inf. Dis. VII, 632 - 640.

ROBERTSON, A. H.
1921 The relation between bacterial counts from milk as obtained by microscopic and plate methods. N. Y. (Geneva)State Agr. Exp. Sta. Tech. Bul. No.86.

ROSELL, L. M.
1933 Le Mammite Streptococcique De La Vache. Ministere De L'Agriculture De La Province De Quebec.

ROSS, H. E.
1912 The Cell content of milk. Jour. of Inf. Dis. X, 7 - 16.

SKAR, O.
1913 Verhalten der Leukozyten der Milch bei der Methylenblaureduktaseprobe. Zeitsch. f. Fleich u. Milchhyg. 23, 442 - 447.

SKAR, O.
1931 Die Reduktaseprobe. Report of the International Dairy Congress. Copenhagen, German edition, Section 2, 58 - 68.

THORNTON, H. R.
1933 The use of the methylene blue reduction test. Can. Pub. Health Jour., XXIV, 192 - 196.

_____
1930 The Reductase test and plate counts. Can. Pub. Health Jour. XXI, 302 - 304.

_____, and HASTINGS, E. G.
1929 Studies on oxidation-reduction in milk. I. Oxidation-reduction potentials and the mechanism of reduction. Jour. Bact., XVIII, 293 - 318.

_____, and HASTINGS, E. G.
1930 Studies on oxidation-reduction in milk. The methylene blue reduction test. Jour. Dai. Sci., XIII, 221 - 245.

_____, and STRYNADKA, N. J.
1934 A gasoline heated cabinet for the sterilization of dairy utensils on the farm. The Can. Dai. and Ice Cream Jour. July.

_____, STRYNADKA, N. J., WOOD, F. W., and ELLINGER, C.
1934 The production of milk of low bacterial content. The Can. Dai. and Ice Cream Jour. August.

_____, STRYNADKA, N. J., WOOD, F.W., and ELLINGER, C.
1934 Milk contamination and the methylene blue reduction test. Can. Pub. Health Jour. XXV, 284 - 294.

TROY, H. C.
1925 A comparison of methylene blue reduction test and the direct microscopic count in grading milk in milk plants. Jour. Dai. Sci., IV, 282 - 285.

TWEED, R. F.
1923 The relation of high cellular counts to Bacterium abortus infection of the udder. Mich. Agr. Exp. Sta., Tech. Bul. No. 61.

UDALL, D. H., and JOHNSON, S.D.
1930 Diagnosis and control of mastitis. Cornell Veterinarian, XXI, 190 - 206.

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T A B L E S

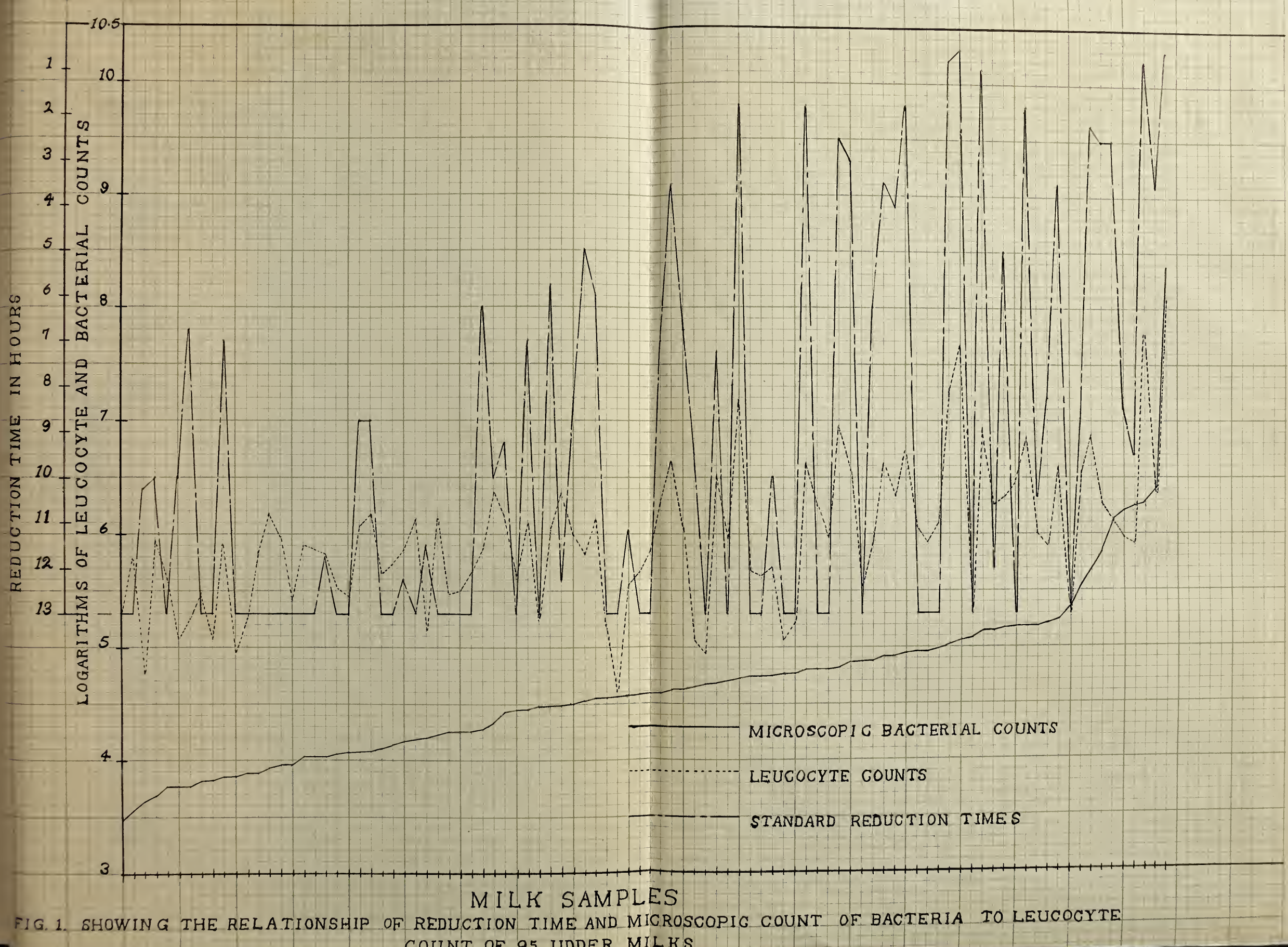

FIG. 1. SHOWING THE RELATIONSHIP OF REDUCTION TIME AND MICROSCOPIC COUNT OF BACTERIA TO LEUCOCYTE COUNT OF 95 UDDER MILKS

TABLE I

PLATE COUNTS AND REDUCTION TIMES OF 56 HERD AND UDDER MILKS

Milk No.	Type of Milk	Standard Red. Time	Plate Count	Milk No.	Type of Milk	Standard Red. Time	Plate Count
1	Udder	0:30	100	29	Herd	6:00	205,500
2	"	0:45	200,500	30	"	6:30	172,000
3	"	0:55	4,500	31	"	6:30	67,500
4	"	1:45	1,000	32	"	6:30	14,500
5	"	1:45	14,150	33	"	6:15	12,000
6	"	2:10	500	34	"	6:45	550
7	Herd	2:30	158,000	35	"	6:45	8,100
8	Udder	3:00	75,250	36	Udder	7:00	17,200
9	Herd	3:12	43,900	37	Herd	7:00	108,000
10	"	3:25	174,000	38	"	7:00	165,000
11	Udder	3:30	2,300	39	"	7:15	12,000
12	"	3:30	511,000	40	"	7:15	51,800
13	Herd	4:00	225,000	41	"	7:15	114,500
14	"	4:10	39,500	42	"	7:30	450
15	"	4:15	101,000	43	"	7:45	400
16	"	4:15	104,000	44	"	8:00	44,000
17	"	4:45	27,500	45	"	7:30	14,675
18	"	4:45	304,000	46	"	8:00	4,200
19	Udder	5:00	7,600	47	Udder	8:15	26,350
20	Herd	5:00	41,650	48	"	8:15	3,400
21	Udder	5:00	100	49	Herd	8:00	44,000
22	Herd	5:10	12,500	50	"	8:45	2,080
23	"	5:15	34,200	51	"	8:45	18,075
24	"	5:15	9,450	52	"	8:40	1,950
25	"	5:15	15,750	53	Udder	8:30	8,350
26	"	5:20	11,225	54	Herd	8:45	28,650
27	"	5:30	70,000	55	"	8:45	38,250
28	"	5:45	29,800	56	Udder	9:00	900

TABLE II

THE REDUCTION TIMES AND PLATE COUNTS OF MORNING MILKS (a) DRAWN INTO STERILE UTENSILS: (b) (a) POURED INTO AND MIXED IN PRODUCER'S CAN: AND (c) MILKED INTO PRODUCER'S UTENSILS

Farm No.	Description of Milk Samples	Reduction Time		Plate Count
		Standard	Modified	
	a	12:05	8:30	1,800
1.	b	9:00	8:00	1,650
	c	8:45	6:00	2,080
	a	14:15	6:45	3,700
2.	b	13:00	6:45	2,000
	c	7:15	5:15	51,500
	a	12:15	8:35	,500
3.	b	12:45	8:10	650
	c	12:45	6:45	500
	a	11:30	9:30	550
4.	b	6:45	5:20	8,500
	c	6:45	5:45	8,100
	a	10:30	7:30	3,100
5.	b	7:30	5:15	8,250
	c	7:30	5:15	8,200
	a	10:00	--	400
6.	b	9:00	--	550
	c	7:30	--	950
	a	12:00	8:30	650
7.	b	11:00	8:15	1,050
	c	5:10	4:50	12,550
	a	15:00	9:30	100
8.	b	15:00	9:30	450
	c	6:45	6:15	550
	a	12:30	8:30	450
9.	b	11:45	8:15	2,400
	c	7:30	6:15	450
	a	13:15	8:55	450
10.	b	12:45	8:40	900
	c	12:45	6:45	500
	a	12:20	8:28	1,170
Average	b	10:51	7:35	2,640
	c	8:16	5:54	8,538

1.					
2.					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
AVERAGE					

TABLE III

REDUCTION TIMES, PLATE COUNTS, AND MICROSCOPIC COUNTS OF HERD MILKS DRAWN INTO UTENSILS OF VARYING DEGREES OF STERILITY

Milk No.	Reduction Times		Plate Count	Microscopic Counts		
	Standard	Modified		Groups	Individuals	At Moment of Reduction
1	2:30	2:25	158,000	140,000	1,360,000	51,360,000
2	3:12	3:12	43,900	190,000	300,000	--
3	3:25	2:35	174,000	100,000	2,060,000	45,000,000
4	4:00	3:45	225,000	130,000	1,710,000	75,840,000
5	4:10	3:50	39,500	170,000	280,000	--
6	4:15	3:50	101,000	100,000	690,000	65,560,000
7	4:15	3:55	104,000	220,000	2,480,000	60,000,000
8	4:45	4:30	27,500	70,000	90,000	72,000,000
9	4:45	4:30	304,000	420,000	860,000	--
10	5:00	4:45	41,650	40,000	70,000	--
11	5:10	4:50	12,550	150,000	230,000	--
12	5:15	4:15	34,200	90,000	1,250,000	--
13	5:15	5:00	9,450		1,000,000	--
14	5:15	5:00	15,750		420,000	--
15	5:20	4:45	11,225	240,000	390,000	--
16	5:30	5:00	70,000	150,000	460,000	66,000,000
17	5:45	4:30	29,800	70,000	180,000	--
18	6:00	4:15	205,500	60,000	740,000	93,600,000
19	6:30	6:30	172,000		290,000	--
20	6:30	5:45	67,500	20,000	80,000	115,200,000
21	6:30	6:00	14,500	60,000	90,000	87,000,000
22	6:15	5:00	12,000	100,000	140,000	165,600,000
23	6:45	6:15	550	10,000	180,000	--
24	6:45	5:45	8,100	40,000	420,000	--
25	7:15	5:45	1,200	30,000	380,000	--
26	7:30	6:30	14,675	20,000	260,000	--
27	7:30	6:15	450	10,000	80,000	--
28	7:15	4:15	114,500	70,000	470,000	90,600,000
29	7:15	5:15	51,800	30,000	160,000	184,200,000
30	12:15	7:45	2,100	100,000	110,000	150,600,000
31	13:00	8:30	150	20,000	20,000	242,400,000
32	13:00	6:45	2,000	50,000	60,000	150,000,000
33	13:42	9:45	450	80,000	80,000	88,200,000
34	15:00	7:45	250	20,000	20,000	166,800,000
35	14:00	10:00	680	10,000	50,000	88,200,000

TABLE 110

RESULTS OF TRIALS, 12-CC ENGINE, AND CORRESPONDING DATUM
OF TANK AIRFLOW GIVEN BY STANDARD AT CARBURETOR
INTAKE OF ORIFICE

TABLE IV

THE MICROSCOPIC BACTERIAL COUNTS
OF 37 UDDER MILKS

Milk No.	Microscopic Counts Based on: 60 fields	1,000 fields	2,000 fields	No. of fields Examined before finding first Bacterium	No. of field in which the largest group was found	No. of cells in the largest group
1.	0	31,200	---	80	---	---
2.	80,000	363,000	---	11	814	232
3.	0	17,400	---	205	---	---
4.	-	35,000	---	83	832	8
5.	0	107,400	---	75	75	97
6.	0	50,400	---	69	967	6
7.	0	6,000	---	131	---	---
8.	40,000	121,200	---	4	87	60
9.	0	9,000	---	110	929	2
10.	10,000	99,600	---	31	886	36
11.	0	12,000	---	171	---	---
12.	10,000	39,600	139,800	57	1,252	148
13.	0	6,000	---	142	---	---
14.	0	7,200	---	189	882	2
15.	0	4,200	---	174	---	---
16.	0	7,800	---	158	---	2
17.	0	12,000	---	69	253	10
18.	370,000	48,600	34,800	47	47	14
19.	0	3,000	---	94	---	---
20.	0	720,000	---	---	465	1,150
21.	0	15,600	---	120	120	6
22.	0	41,400	---	---	80	50
23.	0	15,600	---	103	---	---
24.	960,000	175,200	---	10	623	110
25.	0	18,000	---	115	312	2
26.	90,000	158,400	---	18	104	150
27.	0	7,800	---	98	281	2
28.	30,000	64,800	147,800	30	1,620	250
29.	0	15,000	---	90	---	---
30.	90,000	63,600	119,700	16	1,114	34
31.	0	18,000	---	80	---	---
32.	90,000	81,600	200,700	29	1,754	350
33.	170,000	115,200	477,000	3	1,093	200
34.	70,000	138,600	142,400	32	65	24
35.	60,000	120,000	146,400	57	1,050	58
36.	20,000	64,200	132,800	30	1,267	150
37.	0	36,000	---	471	---	---

TABLE V

THE MICROSCOPIC BACTERIAL COUNTS OF 8 REPLICATE SMEARS FROM EACH OF 2 UDDER MILKS, BASING EACH COUNT UPON THE TOTAL BACTERIA IN AN ENTIRE SMEAR

Smear No.	Milk No. 1 — Microscopic Counts of Bacteria			Milk No. 2 — Microscopic Counts of Bacteria		
	Largest Group Observed	Groups	Individual Cells	Largest Group Observed	Groups	Individual Cells
1.	2 cells	8,400	9,300	28 cells	17,900	25,400
2.	16 cells	10,800	13,200	18 cells	29,000	38,500
3.	200 cells	9,200	30,600	50 cells	23,400	53,500
4.	22 cells	9,200	12,900	8 cells	21,600	28,600
5.	60 cells	8,400	17,600	6 cells	32,600	38,600
6.	4 cells	10,600	12,600	50 cells	41,500	60,100
7.	4 cells	8,800	12,800	6 cells	37,300	44,800
8.	10 cells	8,100	10,400	10 cells	42,200	46,400
Mean		9,187.5	14,925		30,687.5	41,987.5
Maximum Deviation from the mean in Per Cent		17.55	105.02		41.63	43.11
Ratio Lowest Count to Highest Count		1:1.33	1:3.29		1:2.35	1:2.36
Average Absolute Deviation from the Mean in Per Cent		6.93	30.73		25.13	21.93
Plate Count			2,750			
Standard Reduction Time			13:00			
Modified Reduction Time			9:30			

TABLE 7

THE RELATIONSHIP TO BACTERIAL COUNTS OF % REDUCTANTS DURING
FOOD RACK OF 2 DOZEN MILKS, BUILT TO EACH SHORT DROP
THE TOTAL BACTERIA IN AT ENTIRE AREAS

Sheet No.	Microscopic Counts of Bacteria					
	Mink No. 1			Mink No. 2		
	Largest Group Observed	Groups	Individual Cells	Largest Group Observed	Groups	Individual Cells
1.	2 cells	8,400	9,300	25 cells	17,900	25,400
2.	15 cells	10,800	15,200	28 cells	24,800	38,500
3.	200 cells	9,200	50,800	30 cells	24,400	37,800
4.	22 cells	2,200	12,700	8 cells	21,600	31,600
5.	50 cells	8,400	17,600	6 cells	32,100	36,100
6.	4 cells	10,000	14,600	30 cells	41,300	63,100
7.	4 cells	1,800	12,100	8 cells	21,300	14,800
8.	10 cells	1,100	10,400	10 cells	42,700	61,400
Mean		6,147.4	14,025		32,647.4	43,847.5
Maximum Deviation from the Mean in Per Cent		17.35	140.02		41.69	43.11
Ratio Largest Count to Highest Count		1:1.53	1:3.69		1:1.49	1:2.48
Average Deviation from the Mean in Per Cent		6.95	70.12		25.13	21.89
Fair Count			7,400			
Standard Deviation from			15:00			
Modified Deviation from			9:30			

TABLE VI

LEUCOCYTE COUNTS FROM REPLICATE SMEARS
BASING EACH COUNT UPON THE EXAMINATION OF 60 FIELDS

Smear No.	Milk No. 1	Milk No. 2	Milk No. 3	Milk No. 4
1.	3,210,000	880,000	140,000	890,000
2.	3,270,000	1,000,000	130,000	620,000
3.	2,250,000	950,000	120,000	710,000
4.	2,570,000	910,000	160,000	800,000
5.	3,690,000	770,000	100,000	680,000
6.	3,335,000	890,000	160,000	540,000
7.	3,160,000	760,000	170,000	710,000
8.	2,520,000	600,000	130,000	580,000
9.	3,140,000	1,050,000	150,000	480,000
10.	2,360,000		120,000	750,000
11.			110,000	470,000
12.			130,000	450,000
13.				550,000
Mean	2,950,500	867,888	135,000	626,923
Maximum Deviation from the mean in per cent	25.05	30.85	25.70	42.07
Ratio Lowest Count to Highest Count	1:1.64	1:1.75	1:1.70	1:1.97
Average Absolute Deviation from the mean in per cent	13.90	12.11	12.96	17.14

TABLE VII

LEUCOCYTE COUNTS OF 7 MILKS
BASING EACH COUNT UPON THE EXAMINATION OF 60 FIELDS,
720 FIELDS PER MILK BEING EXAMINED

Count No.	Milk No.1	Milk No.2	Milk No.3	Milk No.4	Milk No.5	Milk No.6	Milk No.7
1.	580,000	4,230,000	370,000	100,000	4,730,000	740,000	1,560,000
2.	1,130,000	3,070,000	220,000	120,000	5,810,000	750,000	1,730,000
3.	1,270,000	3,100,000	340,000	60,000	5,780,000	740,000	1,900,000
4.	1,290,000	3,430,000	390,000	50,000	6,590,000	1,030,000	2,120,000
5.	1,510,000	4,010,000	310,000	80,000	4,910,000	810,000	2,150,000
6.	1,360,000	4,630,000	280,000	130,000	5,780,000	1,060,000	1,850,000
7.	1,430,000	3,980,000	300,000	90,000	5,090,000	700,000	1,700,000
8.	650,000	3,390,000	250,000	50,000	4,910,000	780,000	1,580,000
9.	1,160,000	4,170,000	430,000	60,000	4,440,000	540,000	1,480,000
10.	1,480,000	4,260,000	340,000	50,000	4,080,000	770,000	1,600,000
11.	1,600,000	4,060,000	300,000	60,000	4,600,000	730,000	1,830,000
12.	1,400,000	3,820,000	340,000	80,000	4,700,000	770,000	1,790,000
Mean	1,238,333	3,845,850	322,500	77,500	5,116,666	783,333	1,774,166
Maximum Deviation from the mean in per cent	53.17	22.80	33.33	67.74	28.54	35.32	21.18
Ratio Lowest Count to Highest Count	1:2.75	1:1.50	1:1.95	1:2.60	1:1.61	1:1.98	1:1.45
Average Absolute Deviation from the mean in per cent	19.29	10.29	14.21	29.03	11.34	11.48	9.34

TABLE VIII

MICROSCOPIC COUNTS OF BACTERIA AND LEUCOCYTES; EXTENT OF CLUMPING OF BACTERIA; PLATE COUNTS; AND THE METHYLENE BLUE REDUCTION TIMES OF 95 UDDER MILKS.

Milk No.	Leucocyte Count (in thousands)	Reduction Time Standard	Reduction Time Modified	Microscopic Count (1,000 fields)	Plate Count	No. of cells in Largest Group	Type of Group
1.	120,000	0:30	0:30	240,000,000‡	7,875,000	too numerous	Chain
2.	58,000	0:45	0:45	1,863,000‡	200,500	53	"
3.	47,400	0:30	0:30	115,200	100	6	Clump
4.	17,880	0:45	-	107,400	--	97	Chain
5.	15,960	1:45	1:45	51,300	1,000	2	diplococci
6.	9,310	2:30	-	64,800	--	50	Clump
7.	8,320	0:55	0:55	138,600	4,500	24	Chain
8.	7,520	2:10	2:15	490,800	500	28	"
9.	7,200	1:45	1:55	158,400	14,150	16	Clump
10.	5,340	1:45	-	88,200	--	14	"
11.	4,430	3:30	-	40,800	--	2	diplococci
12.	4,160	1:45	-	63,600	--	4	Clump
13.	4,150	3:30	3:30	81,600	2,300	10	Chain
14.	4,040	3:30	-	175,200	--	110	"
15.	3,700	3:00	2:30	72,600	75,250	20	"
16.	3,500	7:15	-	48,000	1;350	12	Clump
17.	3,450	8:30	6:45	363,000	8,350	32	Chain
20.	3,000	*14:00	12:50	155,400	1,150	2	diplococci
22.	2,400	*10:00	*10:00	21,000	3,920	1	singlecocci
23.	2,360	3:30	2:45	2,562,000	511,000	250	Chain
24.	2,310	12:15	11:30	31,200	1,250	2	diplococci
26.	2,100	5:00	7:15	154,200	7,600	146	Chain
25.	2,140	4:00	--	82,200	--	4	"
28.	1,960	7:00	-	38,400	--	35	"
29.	1,940	2:30	-	720,000	--	800	"
30.	1,930	12:00	-	140,400	--	8	"
35.	1,740	*15:00	10:45	64,200	1,700	6	Clump
36.	1,570	8:45	8:15	12,000	2,800	6	Chain
37.	1,500	*13:00	-	8,400	--	2	diplococci
39.	1,458	2:30	2:45	1,383,600	273,000	200	Chain
40.	1,440	*13:00	-	17,000	--	2	diplococci
41.	1,430	9:15	8:05	26,400	600	2	"
43.	1,320	6:00	-	34,800	--	4	Chain
44.	1,300	*13:00	-	15,600	--	1	singlecocci
45.	1,270	13:00	6:00	99,600	4,400	32	Chain
46.	1,270	7:00	6:30	28,200	17,200	8	"
47.	1,190	6:45	-	41,400	--	50	"
48.	1,170	8:45	-	12,000	--	1	singlecocci
50.	1,170	13:20	9:30	90,000	2,600	8	Chain
52.	1,060	5:45	-	31,200	--	1	singlecocci
54.	1,030	10:25	9:00	158,400	24,950	150	Chain
57.	1,000	8:15	7:45	31,800	26,350	24	"
58.	990	*13:00	-	9,000	--	2	diplococci
60.	960	8:30	3:30	1,593,600	63,600	92	chain
61.	940	14:00	10:55	64,200	7,850	8	"
62.	930	14:15	8:15	50,400	2,000	6	Clump
64.	900	*10:00	*10:00	4,800	--	1	singlecocci
65.	900	7:00	-	7,200	--	1	"
66.	860	6:15	14:45	75,000	50	32	Clump
67.	840	*15:00	8:05	91,200	150	2	diplococci
68.	840	9:30	3:30	1,799,400	28,750	800	Chain
70.	800	8:15	6:00	163,800	3,400	12	Clump
71.	800	*13:00	-	10,800	--	1	singlecocci
72.	790	*13:00	-	10,800	--	2	diplococci
73.	780	6:15	-	19,200	--	10	Chain
74.	770	*15:00	12:45	30,600	600	2	diplococci
75.	750	12:15	-	15,000	--	1	singlecocci
79.	710	*14:00	8:40	38,400	3,400	3	Clump
81.	680	5:00	4:45	33.600	100	2	diplococci
82.	660	11:45	9:15	10,800	1,000	2	"
83.	660	*13:00	-	7,800	--	2	"
85.	600	*13:00	-	3,600	--	2	"
88.	540	*13:00	-	13,800	--	1	singlecocci
92.	520	10:00	7:00	55,800	7,750	16	Chain
93.	500	13:40	9:15	54,000	1,550	4	"
94.	500	*14:00	8:45	38,400	550	2	diplococci
96.	470	*13:00	-	18;000	--	2	"
98.	450	*13:00	-	12,600	--	1	singlecocci
102.	430	*13:00	-	6,000	--	2	diplococci
104.	420	*15:00	12:15	27,600	750	2	"
105.	420	*15:00	7:30	54,000	950	6	Clump
109.	360	11:10	8:15	37,000	650	2	diplococci

92.	520	10:00	7:00	55,800	7,750	16	Chain
93.	500	13:40	9:15	54,000	1,550	4	"
94.	500	*14:00	8:45	38,400	550	2	diplococci
96.	470	*13:00	-	18,000	--	2	"
98.	450	*13:00	-	12,600	--	1	singlecocci
102.	430	*13:00	-	6,000	--	2	diplococci
104.	420	*15:00	12:15	27,600	750	2	"
105.	420	*15:00	7:30	54,000	950	6	Clump
109.	360	11:10	8:15	37,200	650	2	diplococci
111.	340	*14:00	13:10	75,000	300	2	"
112.	340	*13:00	-	11,400	--	6	Clump
118.	320	*13:00	-	18,000	--	1	singlecocci
119.	310	*13:00	-	6,600	--	1	"
120.	310	*13:00	-	18,000	--	1	"
122.	290	*13:00	-	12,000	--	1	"
126.	260	*13:00	-	9,000	--	2	diplococci
130.	210	*14:00	10:40	121,200	150	87	Clump
132.	200	*13:00	-	3,000	--	1	singlecocci
133.	200	*24:00	12:30	231,600	350	28	Chain
135.	180	*13:00	-	7,800	--	1	singlecocci
136.	180	6:45	-	6,000	--	1	"
137.	170	*15:00	12:45	58,800	600	16	Chain
138.	160	*14:00	13:10	35,400	1,100	6	"
143.	140	11:30	10:15	16,200	250	2	diplococci
146.	120	*15:00	12:45	57,000	750	2	"
149.	120	10:00	9:30	6,000	100	1	singlecocci
150.	120	9:30	9:00	43,200	4,600	8	Chain
151.	120	*13:00	-	6,600	--	1	singlecocci
154.	90	*13:00	-	7,200	--	2	diplococci
155.	90	*15:00	12:15	47,400	150	2	"
158.	60	10:15	9:45	4,200	2,700	1	singlecocci
162.	40	*15:00	13:30	36,000	100	2	diplococci

† In milks No. 1 and No. 2, 5 and 200 fields per smear were searched respectively.

* Means 'greater than'

TABLE IX

LEUCOCYTE COUNTS, PLATE COUNTS, AND METHYLENE BLUE REDUCTION TIMES OF 163 UDDER MILKS

Milk No.	Leucocyte Count (in thousands)	Reduction Time		Plate Count	Milk No.	Leucocyte Count (in thousands)	Reduction Time		Plate Count
		Standard	Modified				Standard	Modified	
1.	120,000	0:30	0:30	7,875,000	83.	660	*13:00	--	--
2.	58,000	0:45	0:45	200,500	84.	630	*15:00	8:15	400
3.	47,400	0:30	0:30	100	85.	600	*13:00	--	--
4.	17,880	0:45	--	--	86.	570	*15:00	*15:00	50
5.	15,960	1:45	1:45	1,000	87.	560	*14:00	12:15	350
6.	9,310	2:30	--	--	88.	540	*13:00	--	--
7.	8,320	0:55	0:55	4,500	89.	530	*13:00	13:00	450
8.	7,520	2:10	2:15	500	90.	530	*14:00	9:00	100
9.	7,200	1:45	1:55	14,150	91.	520	9:15	--	4,400
10.	5,340	1:45	--	--	92.	520	10:00	7:00	7,750
11.	4,430	3:30	--	--	93.	500	13:40	9:15	1,550
12.	4,160	1:45	--	--	94.	500	*15:00	8:45	550
13.	4,150	3:30	3:30	2,300	95.	470	*13:00	*13:00	50
14.	4,040	3:30	--	--	96.	470	*13:00	--	--
15.	3,700	3:00	2:30	75,250	97.	460	12:15	--	--
16.	3,500	7:15	--	1,350	98.	450	*13:00	--	--
17.	3,450	8:30	6:45	8,350	99.	450	*14:00	8:45	200
18.	3,140	*14:00	--	100	100.	440	*14:00	12:15	500
19.	3,130	*24:00	--	100	101.	430	*14:00	8:00	450
20.	3,000	*14:00	12:50	1,150	102.	430	*13:00	--	--
21.	2,570	*13:00	9:30	500	103.	430	*14:00	--	350
22.	2,400	*10:00	*10:00	3,920	104.	420	*15:00	12:15	750
23.	2,360	3:30	2:45	511,000	105.	420	*15:00	7:30	950
24.	2,310	12:15	11:30	1,250	106.	420	13:45	8:45	1,350
25.	2,140	4:00	--	--	107.	380	*14:00	10:15	1,250
26.	2,100	5:00	7:15	7,600	108.	370	*14:00	8:30	150
27.	2,090	13:00	6:00	2,500	109.	360	11:10	8:15	650
28.	1,960	7:00	--	--	110.	350.	*13:00	*13:00	100
29.	1,940	2:30	--	--	111.	340.	*14:00	13:10	300
30.	1,930	12:00	--	--	112.	340	*13:00	--	--
31.	1,910	10:30	8:30	--	113.	340	*15:00	10:00	50
32.	1,860	9:00	7:30	900	114.	330	*16:00	9:30	2,130
33.	1,800	14:00	8:30	650	115.	320	*14:00	--	800
34.	1,800	*14:00	10:35	350	116.	320	12:45	--	650
35.	1,740	*15:00	10:45	1,700	117.	320	11:15	10:15	150
36.	1,570	8:45	8:15	2,800	118.	320	*13:00	--	--
37.	1,500	*13:00	--	--	119.	310	*13:00	--	--
38.	1,460	*13:00	9:30	1,050	120.	310	*13:00	--	--
39.	1,450	3:30	2:45	273,000	121.	300	*15:00	11:30	350
40.	1,440	*13:00	--	--	122.	290	*13:00	--	--
41.	1,430	9:15	8:05	600	123.	280	*14:00	--	17,550
42.	1,380	9:45	8:45	450	124.	280	11:15	--	4,450
43.	1,320	6:00	--	--	125.	280	*13:00	--	--
44.	1,300	*13:00	--	--	126.	260	*13:00	--	--
45.	1,270	13:00	6:00	4,400	127.	240	*16:00	13:00	535
46.	1,270	7:00	6:30	17,200	128.	220	12:45	10:00	250
47.	1,190	6:45	--	--	129.	220	*13:00	*13:00	400
48.	1,170	8:45	--	--	130.	210	*14:00	10:40	150
49.	1,170	14:45	8:45	500	131.	210	*15:00	11:30	100
50.	1,170	13:30	9:30	2,600	132.	200	*13:00	--	--
51.	1,140	*14:00	14:30	50	133.	200	*24:00	12:30	350
52.	1,060	5:45	--	--	134.	190	*14:00	*14:00	1,900
53.	1,050	13:00	9:30	2,750	135.	180	*13:00	--	--
54.	1,030	10:25	9:00	24,950	136.	180	6:45	--	--
55.	1,030	9:00	--	2,750	137.	170	*15:00	12:45	600
56.	1,010	9:00	--	250	138.	160	*14:00	13:10	1,100
57.	1,000	8:15	7:45	26,350	139.	160	*13:00	10:45	200
58.	990	*13:00	--	--	140.	160	13:15	--	1,200
59.	900	*14:00	--	2,750	141.	140	*14:00	--	950
60.	960	8:30	3:30	63,600	142.	140	*14:00	--	3,950
61.	940	14:00	10:55	7,850	143.	140	11:30	10:15	250
62.	930	14:15	8:15	2,000	144.	130	*14:00	--	150
63.	920	*13:00	*13:00	100	145.	130	*16:00	11:30	80
64.	900	*10:00	*10:00	200	146.	120	*15:00	12:45	750
65.	900	7:00	--	--	147.	120	*13:00	*13:00	100
66.	860	6:15	14:45	50	148.	120	14:15	10:00	450
67.	840	*15:00	8:05	150	149.	120	10:00	9:30	100
68.	840	9:30	3:30	28,750	150.	120	9:30	9:00	4,600
69.	810	*14:00	11:15	300	151.	120	*13:00	--	--
70.	800	8:15	6:00	3,400	152.	100	*15:00	11:00	100
71.	800	*13:00	--	--	153.	100	*16:00	14:15	70
72.	790	*13:00	--	--	154.	90	*13:00	--	--
73.	780	6:15	--	--	155.	90	*15:00	12:15	150
74.	770	*15:00	12:45	600	156.	80	*16:00	15:45	25
75.	750	12:15	--	--	157.	60	*14:00	--	150
76.	7 730	*14:00	11:00	1,600	158.	60	10:15	9:45	2,700
77.	720	*15:00	10:30	150	159.	50	*14:00	11:15	450
78.	710	*16:00	13:45	5	160.	50	*15:00	11:45	100
79.	710	*14:00	8:40	3,400	161.	40	12:45	8:55	150
80.	708	*14:00	11:30	50	162.	40	*15:00	13:30	100
81.	680	5:00	4:45	100	163.	30	*15:00	11:45	50
82.	660	11:45	9:15	1,000					

TABLE X

LEUCOCYTE COUNTS AND THE MICROSCOPIC BACTERIAL COUNTS IN MILK
RELATED TO THE MICROSCOPIC BACTERIAL COUNTS AT THE MOMENT OF REDUCTION
OF THE METHYLENE BLUE IN MILK

Milk No.	Reduction Time		Leucocyte Count (in thousands)	Plate Count	Initial Microscopic Bacterial Count	Fields Searched per Smear	Microscopic Bacterial Count at Reduction	Fields Searched per Smear
	Standard	Modified						
3.	0:30	0:30	47,400	100	477,000	2,000	68,400	2,000
1.	0:30	0:30	120,000	7,875,000	240,000,000	5	204,000,000	5
4.	0:45	---	17,880	--	107,400	1,000	253,800†	2,000
7.	0:55	0:55	8,320	4,500	142,400	2,000	86,000	2,000
12.	1:45	----	4,160	---	119,700	2,000	364,200†	2,000
10.	1:45	---	5,340	---	91,200	2,000	59,700†	2,000
5.	1:45	1:45	15,960	1,000	48,000	2,000	43,200	2,000
8.	2:10	2:15	7,520	500	335,400	2,000	146,400	2,000
13.	3:30	3:30	4,150	2,300	200,700	2,000	56,400	2,000
81.	5:00	4:45	680	100	39,200	2,000	81,600	2,000
26.	5:00	7:15	2,100	7,600	143,100	2,000	1,017,600†	1,000
66.	6:15	14:45	860	50	75,000	1,000	132,800†	2,000
16.	7:15	---	3,500	1,350	56,100	2,000	435,600†	1,000
70.	8:15	6:00	800	3,400	163,800	1,000	174,600,000	5
60.	8:30	3:30	960	63,600	1,593,600	1,000	228,600,000	5
68.	9:30	3:30	840	28,750	1,799,400	1,000	177,600,000	5
92.	10:00	7:00	520	7,750	55,800	1,000	90,000,000	5
109.	11:10	8:15	360	650	37,200	1,000	150,000,000	5
45.	13:00	6:00	1,270	4,400	99,600	1,000	205,800,000	5
93.	13:40	9:15	500	1,550	54,000	1,000	108,000,000	5
61.	14:00	10:55	940	7,850	64,200	1,000	92,000,000	5
50.	13:30	9:30	1,170	2,600	90,000	1,000	165,200,000	5
62.	14:15	8:15	930	2,000	50,400	1,000	189,000,000	5
79.	*14:00	8:40	710	3,400	38,400	1,000	102,000,000	5
130.	*14:00	10:40	210	150	121,200	1,000	96,000,000	5
111.	*14:00	13:10	340	300	75,000	1,000	120,000,000	5
138.	*14:00	13:10	160	1,100	35,400	1,000	100,000,000	5
35.	*15:00	10:45	1,740	1,700	64,200	1,000	91,200,000	5
137.	*15:00	12:45	170	600	58,800	1,000	132,000,000	5
67.	*15:00	8:05	840	150	91;200	1,000	285,600,000	5
94.	*15:00	8:45	500	550	38,400	1,000	72,000,000	5
104.	*15:00	12:15	420	750	27,600	1,000	87,000,000	5
74.	*15:00	12:45	770	600	30,600	1,000	95,400,000	5
155.	*15:00	12:15	90	150	47,400	1,000	155,400,000	5
162.	*15:00	13:30	40	100	36,000	1,000	67,800,000	5
146.	*15:00	12:45	120	750	57,000	1,000	90,600,000	5
105.	*15:00	7:30	420	950	54,000	1,000	157,200,000	5
133.	*24:00	12:30	200	350	231,600	1,000	120,000,000	5
55.	9:00	---	1,030	2,750	---	---	94,800,000†	5
56.	9:00	---	1,010	250	---	---	180,000,000†	5
39.	9:00	7:30	1,860	900	---	---	132,000,000	5
27.	13:00	6:00	2,090	2,500	---	---	180,000,000	5
53.	13:00	9:30	1,050	2,750	---	---	69,000,000	5
76.	*14:00	11:00	730	1,600	---	---	144,000,000	5
100.	*14:00	12:15	440	500	---	---	108,600,000	5
67.	*14:00	12:15	560	350	---	---	109,200,000	5

Note: † These microscopic bacterial counts at the moment of reduction were obtained from the Standard tests.

* means 'greater than'

TABLE XI

BACTERIAL COUNTS OF MILK AFTER THE PROLONGED INCUBATION OF THE REDUCED SAMPLES

Milk No.	Reduction Time		Additional Period of Incubation after Reduction	Microscopic Count after 8 hours Incubation	Fields Per Smear Examined	Reduction Technique Tube used For Smears	Plate Count after 8 hrs. Incubation
	Standard	Modified					
3.	0:30	0:30	7:30	590,000	60	Modified	--
7.	0:55	0:55	7:05	670,800,000	5	"	--
5.	1:45	1:45	6:15	6,000,000	60	"	--
8.	2:10	2:15	5:45	920,000	60	"	900,000
13.	3:30	3:30	4:30	140,000	60	"	--
81.	5:00	4:45	3:15	210,000	60	"	32,000
26.	5:00	7:15	3:00	18,660,000	60	Standard	--
66.	6:15	14:45	1:45	350,000	60	"	60,000

TABLE 13

DISTRIBUTION OF DEATHS BY CAUSE AND SEX

Cause of death	Total		Male		Female	
	Number	Rate	Number	Rate	Number	Rate
All causes	1,234	12.3	1,234	12.3	1,234	12.3
Cardiovascular diseases	1,234	12.3	1,234	12.3	1,234	12.3
Respiratory diseases	1,234	12.3	1,234	12.3	1,234	12.3
Digestive diseases	1,234	12.3	1,234	12.3	1,234	12.3
Infectious diseases	1,234	12.3	1,234	12.3	1,234	12.3
Accidents	1,234	12.3	1,234	12.3	1,234	12.3
Other causes	1,234	12.3	1,234	12.3	1,234	12.3

TABLE XII

METHYLENE BLUE REDUCTION TIMES OF
UDDER MILK PLUS BOVINE BLOOD LEUCOCYTES

Tube No.	Description of Samples	Reduction Time		Microscopic Bacterial Count at Reduction
		Standard	Modified	
1.	Milk only	25:30	12:30	
2.	" "	24:45	12:45	
3.	" "	25:45	13:15	
4.	9 c.c. milk and 1 c.c. Leuc. Susp.	13:30	11:00	200,000,000
5.	9 c.c. milk and 1 c.c. from Tube 4	16:00	11:45	
6.	9 c.c. milk and 1 c.c. from Tube 5	16:00	12:15	
7.	9 c.c. milk and 1 c.c. Leuc. Susp.	12:30	10:45	190,000,000
8.	9 c.c. milk and 1 c.c. from Tube 7	16:30	11:30	
9.	9 c.c. milk and 1 c.c. from Tube 8	16:30	12:45	
10.	9 c.c. milk and 1 c.c. Blood Serum	12:15	12:00	120,000,000
11.	9 c.c. milk and 1 c.c. from Tube 10	17:00	12:00	
12.	9 c.c. milk and 1 c.c. from Tube 11	18:15	12:45	
13	9 c.c. milk and 1 c.c. Red Cell Susp.	12:15	11:00	126,000,000
14.	9 c.c. milk and 1 c.c. from Tube 13	13:15	12:30	
15.	9 c.c. milk and 1 c.c. from Tube 14	19:15	12:30	

Note: A leucocyte suspension of 500 million cells per c.c. gives an approximate count of 50 million leucocytes per c.c. in Tube No. 4. The milk used contained 330,000 leucocytes per c.c.

TABLE 221

Item No.	Description of Machine	Production Time		Electrical Energy Consumption
		Standard Machine	Automatic Machine	
1.	Milk cans	45:37	12:30	
2.	" "	24:45	12:45	
3.	" "	27:45	13:15	
4.	1 c.c. milk can 1 c.c. Lens. Spec.	42:30	12:00	200,000,000
5.	1 c.c. milk can 1 c.c. Lens Tube 4	28:00	12:15	
6.	1 c.c. milk can 1 c.c. Lens Tube 7	26:00	12:30	
7.	1 c.c. milk can 1 c.c. Lens. Spec.	43:30	12:00	175,000,000
8.	1 c.c. milk can 1 c.c. Lens Tube 7	26:30	12:30	
9.	1 c.c. milk can 1 c.c. Lens Tube 8	28:30	12:45	
10.	1 c.c. milk can 1 c.c. Thread Spring	54:15	12:00	150,000,000
11.	1 c.c. milk can 1 c.c. Lens Tube 12	31:00	12:30	
12.	1 c.c. milk can 1 c.c. Lens Tube 15	31:30	12:45	
13.	1 c.c. milk can 1 c.c. Pen Cell Body.	47:00	12:00	175,000,000
14.	1 c.c. milk can 1 c.c. Lens Tube 19	31:00	12:45	
15.	1 c.c. milk can 1 c.c. Lens Tube 14	31:15	12:30	

Note: A Jacobette suggestion is that milk cans of 4 litres or approximate usual be 10 milk tins per c.c. 12 Tins Ex. 4. The milk used ... Jacobette per c.c.

TABLE XIII

METHYLENE BLUE REDUCTION TIMES OF UDDER MILK PLUS RABBIT LEUCOCYTES

Tube No.	Description of Samples	Reduction Time Standard	Reduction Time Modified	Leucocyte Count	Microscopic Bacterial Count at Reduction
1.	45 c.c. milk plus	more	11:00	1,120,000	66,400,000
2.	10 c.c. Leuc.Susp.	than	12:00		
3.	45 c.c. milk plus	16:00	11:00	730,000	68,400,000
4.	7 c.c. Leuc.Susp.	"	12:00		
5.	45 c.c. milk plus	"	11:00	590,000	77,200,000
6.	3 c.c. Leuc.Susp.	"	11:00		
7.	45 c.c. milk plus	"	10:30	520,000	70,400,000
8.	0.5 c.c. Leuc.Susp.	"	10:30		
9.	Milk only	"	10:00	390,000	
10.		"	10:30	290,000	
11.	45 c.c. milk plus	"	11:00		84,000,000
12.	10 c.c. saline	"	11:00		
13.	45 c.c. milk plus	"	10:30		
14.	5 c.c. saline	"	11:00		

Note: Leucocyte suspension used in above experiment contained approximately 10 million cells per cubic centimeter. The leucocyte counts reported above were obtained from smears made after the leucocyte suspension was added to milk.

TABLE XIV

LEUCOCYTE COUNTS AND METHYLENE BLUE REDUCTION TIMES OF MILK FROM INDIVIDUAL QUARTERS OF 27 COWS DRAWN ON DIFFERENT DAYS

Cow No.	Teat	Leucocyte Count (In Thousands)			Reduction Time Standard			Reduction Time Modified		
	Survey No.	1	2	3	1	2	3	1	2	3
1.	R.F.	200	1,250	180	18:00	16:45	*24:00	8:15	9:30	8:30
	L.F.	270	1,310	520	18:00	14:30	16:45	7:30	7:00	8:30
	R.R.	350	70	510	25:00	16:45	17:45	9:15	9:30	7:15
	L.R.	460	1,500	670	13:45	1:00	11:00	6:30	3:00	7:15
2.	R.F.	880	630	340	18:00	20:00	20:00	8:30	7:00	6:30
	L.F.	4,620	2,590	1,300	1:15	25:00	11:45	1:15	9:30	7:15
	R.R.	440	640	800	18:00	25:00	17:00	8:30	9:30	8:30
	L.R.	1,140	1,300	490	14:30	18:00	13:00	8:30	14:30	8:00
3.	L.F.	270	370	150	21:00	22:00	14:30	9:30	9:30	11:00
	R.R.	100	120	140	10:15	10:30	21:45	9:15	9:30	9:15
	L.R.	110	570	240	14:30	15:15	*24:00	9:30	10:45	12:30
4.	R.F.	170	260	100	16:00	10:30	16:15	7:30	6:00	8:00
	L.F	2,590	2,770	6,880	4:30	7:30	0:30	3:00	4:15	0:30
	R.R.	190	1,100	160	13:45	20:00	12:30	6:45	10:00	6:30
	L.R.	670	660	1,850	12:00	20:00	4:45	9:30	4:15	3:30
5.	R.F.	180	280	190	16:00	15:15	17:45	8:30	9:30	9:00
	L.F.	10	80	110	13:00	21:30	17:30	9:45	9:30	9:00
	R.R.	210	250	720	16:00	21:30	17:30	7:30	7:00	6:30
	L.R.	30	10	60	19:15	*25:00	20:30	13:00	10:30	10:45
6.	R.F.	20	140	490	14:30	15:45	11:00	10:00	14:00	12:30
	L.F.	40	80	30	13:00	21:00	22:15	9:20	13:00	13:15
	R.R.	130	400	260	12:00	24:30	*24:00	10:30	11:45	13:15
	L.R.	50	80	70	16:00	*25:00	22:15	12:00	13:00	10:45
7.	R.F.	170	1,140	250	21:00	24:30	19:30	11:00	11:30	11:00
	L.F.	120	1,110	210	19:30	20:00	16:30	11:45	10:00	10:15
	R.R.	510	620	560	16:30	15:15	13:00	10:15	9:30	9:00
	L.R.	240	710	260	24:00	17:00	17:45	11:45	9:30	9:15
8.	R.F.	1,010	5,100	1,100	15:45	3:00	11:45	9:00	1:15	7:15
	L.F.	520	70	630	16:30	25:00	12:30	8:15	10:00	8:30
	R.R.	360	1,110	530	18:00	25:00	13:00	9:15	10:00	10:15
	L.R.	110	240	220	19:30	17:00	12:30	11:45	9:30	8:00
9.	R.F.	110	250	400	16:30	16:45	17:30	9:15	13:30	9:15
	L.F.	70	260	80	16:30	*25:00	14:45	10:00	13:00	10:45
	R.R.	100	70	120	15:00	12:30	15:30	9:15	8:30	10:45
	L.R.	30	40	150	23:00	24:30	20:30	10:30	11:45	11:00
10.	R.F.	280	300	140	24:00	24:00	*24:00	13:00	14:30	12:30
	L.F.	150	250	500	18:00	22:00	20:30	13:00	11:30	11:00
	R.R.	550	1,250	420	16:00	25:00	12:30	8:15	17:00	10:45
	L.R.	460	310	440	*24:00	19:00	11:45	14:30	11:45	12:30
11.	R.F	1,050	1,330	1,870	5:30	3:00	11:45	5:45	1:00	7:15
	L.F.	470	420	720	3:30	25:00	11:45	9:30	11:45	8:00
	R.R.	710	720	1,010	10:15	11:45	11:45	7:30	7:30	6:30
	L.R.	250	660	780	16:00	12:30	11:45	11:45	10:45	8:30
12.	R.F.	300	220	340	14:30	17:00	12:30	10:00	7:30	11:00
	L.F.	470	210	250	13:00	12:30	6:30	10:15	9:30	11:00
	R.R.	130	190	570	15:30	16:00	10:15	11:45	10:15	8:30
	L.R.	50	450	720	12:30	15:45	6:30	7:30	9:30	10:15
13.	R.F.	760	600	600	13:00	16:30	16:45	10:15	11:00	7:15
	L.F.	30	210	60	15:00	15:00	14:30	9:30	9:30	8:30
	R.R.	20	30	80	13:00	12:15	13:00	8:15	10:00	7:15
	L.R.	940	670	760	10:00	12:30	6:30	7:30	9:30	10:15
14.	R.F.	100	240	150	21:30	22:00	20:30	10:00	10:45	10:15
	L.F.	50	90	40	15:15	13:00	17:45	8:30	7:00	8:00
	R.R.	70	80	90	13:00	20:00	23:30	7:30	10:30	11:00
	L.R.	170	100	60	14:30	18:30	20:30	11:45	10:45	10:15
15.	R.F.	790	970	1,360	21:30	25:00	23:30	8:15	10:45	12:30
	L.F.	1,040	1,260	770	19:45	*25:00	*24:00	9:15	10:30	11:45
	R.R.	1,150	1,030	610	21:45	*25:00	15:15	9:30	10:30	10:45
	L.R.	930	1,070	590	12:00	22:30	17:15	10:00	10:30	9:15
16.	R.F.	1,670	1,010	1,990	10:00	7:30	6:30	10:00	4:15	13:15
	L.F.	250	190	120	12:30	17:15	19:30	10:00	11:30	12:30
	R.R.	90	70	160	15:45	15:00	14:30	11:45	11:30	10:15
	L.R.	160	120	100	15:00	12:45	11:45	12:00	10:15	8:30

15.	R.F.	790	970	1,360	21:30	25:00	23:30	8:15	10:45	12:30
	L.F.	1,040	1,260	770	19:45	*25:00	*24:00	9:15	10:30	11:45
	R.R.	1,150	1,030	610	21:45	*25:00	15:15	9:30	10:30	10:45
	L.R.	930	1,070	590	12:00	22:30	17:15	10:00	10:30	9:15
16.	R.F.	1,670	1,010	1,990	10:00	7:30	6:30	10:00	4:15	13:15
	L.F.	250	190	120	12:30	17:15	19:30	10:00	11:30	12:30
	R.R.	90	70	160	15:45	15:00	14:30	11:45	11:30	10:15
	L.R.	160	120	100	15:00	12:45	11:45	12:00	10:15	8:30
17.	R.F.	220	80	140	16:30	17:30	16:30	9:30	13:00	10:15
	L.F.	600	390	690	12:30	11:45	12:30	5:30	6:00	8:00
	R.R.	340	200	180	13:00	11:45	14:30	9:15	10:00	10:15
	L.R.	240	160	170	14:30	13:15	16:15	9:15	8:30	8:30
18.	R.F.	1,270	540	390	18:00	22:00	20:30	9:30	10:00	11:00
	L.F.	3,340	2,080	620	15:00	*25:00	21:45	6:00	10:30	11:45
	R.R	250	210	60	13:45	11:45	23:30	8:15	10:45	11:00
	L.R.	490	630	280	15:45	24:00	16:45	8:30	11:30	8:30
19.	R.F	1,840	410	720	6:30	10:45	9:15	7:00	8:30	6:30
	L.F.	2,000	1,570	1,320	5:30	13:30	7:30	4:30	8:30	3:30
	R.R.	70	190	110	12:30	15:45	15:45	9:15	7:30	8:30
	L.R.	1,740	1,540	1,380	10:00	12:30	3:30	5:30	7:30	3:00
20.	R.F.	60	140	60	20:00	24:30	20:30	11:45	14:00	9:00
	L.F.	90	110	130	15:45	22:00	17:45	12:00	12:30	12:30
	R.R.	340	630	580	13:45	6:00	17:45	9:30	5:00	10:15
	L.R.	50	100	70	16:15	18:15	13:30	9:30	10:30	10:15
21.	R.F.	360	290	80	18:00	14:00	20:30	10:45	11:30	10:15
	L.F.	860	750	640	10:00	11:45	10:15	6:30	7:00	8:30
	R.R.	260	160	130	18:00	*25:00	13:00	10:00	11:30	9:15
	L.R.	60	230	90	16:45	17:00	16:30	9:30	11:30	9:00
22.	R.F.	250	490	290	21:00	16:00	20:30	16:00	10:45	12:30
	L.F.	4,210	5,560	2,880	1:15	5:00	12:30	1:30	3:00	6:30
	R.R.	1,350	840	970	5:30	14:30	17:00	10:30	9:30	11:00
	L.R.	1,380	690	370	16:00	*25:00	18:00	11:00	11:30	9:00
23.	R.F.	40	10	50	22:00	20:00	*24:00	12:15	11:30	11:00
	L.F.	20	40	50	19:30	11:45	*24:00	12:00	15:45	12:30
	R.R.	100	190	390	23:00	24:00	23:30	10:00	11:45	11:10
	L.R.	40	30	210	21:00	*25:00	20:30	11:45	16:30	10:15
24.	R.F.	760	250	320	11:00	24:00	20:00	9:30	10:45	10:15
	L.F.	300	520	210	16:30	16:45	20:30	10:00	10:00	10:45
	R.R.	530	350	370	16:00	*25:00	21:45	11:00	11:30	11:45
	L.R.	260	160	130	13:45	25:00	23:30	15:30	12:30	11:45
25.	R.F.	580	340	540	15:00	13:00	20:00	14:30	9:30	11:45
	R.R.	6,280	1,470	1,000	2:00	8:30	8:00	2:00	7:00	7:15
	L.R.	710	6,360	430	8:15	2:00	20:30	5:30	1:00	10:45
26.	L.F.	660	780	3,800	9:15	10:30	10:15	8:30	8:30	10:15
	R.R.	570	910	2,600	8:15	11:45	14:30	8:15	9:30	11:00
	L.R.	460	350	510	8:15	10:30	14:30	8:30	8:30	10:15
27.	R.F.	820	690	360	19:15	18:00	16:30	10:45	13:00	11:00
	L.F.	820	2,530	1,180	13:45	*25:00	*24:00	6:30	16:30	9:00
	R.R.	790	830	300	18:00	10:30	*24:00	12:00	9:30	10:15

Note: The following abbreviations, R.F., L.F., R.R., and L.R. mean respectively: right front, left front, right rear, and left rear.

* Means 'greater than'.

TABLE XV

THE INCIDENCE OF LONGCHAIN STREPTOCOCCI, SHORT REDUCTION TIMES AND HIGH LEUCOCYTE COUNTS IN UDDER MILK FROM 149 COWS IN 13 HERDS

Herd No.	No. of Cows Examined	Number of Cows with Leucocyte Counts Greater than 500,000						Number of Cows with Leucocytes Counts less than 500,000				Cows Classed Abnormal on the Basis of 1 or all 3 Tests	
		Leucocytes alone		Plus Streptococci in 60 fields per smear		Plus Standard Reduction times of 10:00 or less		Plus Standard Reduction times of 10:00 or less		Plus Streptococci in 60 fields per smear			
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	3	3	100.0			1	33.3					3	100.0
2	3	1	33.3									1	33.3
3	9	6	66.6			1	11.1					6	66.6
4	4	2	50.0	1	25.0	1	25.0	2	50.0	1	25.0	4	100.0
5	8	8	100.0	5	62.5	7	87.5					8	100.0
6	42	26	61.9	8	19.0	15	35.7	1	2.3			27	64.2
7	10	4	40.0									4	40.0
8	6	1	16.6									1	16.1
9	8	3	37.5			1	12.5					3	37.5
10	4	3	75.0									3	75.0
11	10	4	40.0			3	30.0					4	40.0
12	32	14	43.7	2	6.2	9	28.1			2	6.2	16	50.0
13	10	5	50.0									5	50.0
	149	80	53.7	16	10.7	38	25.4	3	2.0	3	2.0	85	57.0

Note: In herd No. 4, 1 milk with leucocyte content less than 500,000 per c.c. both harboured Streptococci and reduced in less than 10:00

THE NEW ZEALAND JOURNAL OF AGRICULTURE, MONTH DAY, YEAR

THE EFFECTS OF [illegible] ON [illegible]

[illegible]

[illegible] of [illegible] with [illegible] and [illegible] [illegible] [illegible]

TABLE XVI

INCIDENCE OF MASTITIS AMONG THE COWS IN 7 HERDS CORRELATED WITH THE BACTERIOLOGICAL QUALITIES OF MIXED HERD MILK PRODUCED IN PRODUCER'S AND STERILE UTENSILS

Herd No.	% of Cows in Herd Showing Evidence of Mastitis (Table XV)	Utensils Used	Reduction Time		Plate Count
			Standard	Modified	
3.	66.6	Sterile	12:00	8:30	650
		Producer's	5:10	4:50	12,550
6.	64.2	Sterile	5:45	4:45	29,800
		Producer's	8:45	7:45	18,075
7.	40.0	Sterile	12.30	8:30	450
		Producer's	6:45	6:15	550
9.	37.5	Sterile	12:10	8:40	1,500
		Producer's	11:55	6:55	1,300
11.	40.0	Sterile	15:00	13:30	550
		Producer's	13:45	8:15	6,000
12.	50.0	Sterile	13:15	8:55	450
		Producer's	11:15	6:45	4,500
13	50.0	Sterile	11:30	9:30	550
		Producer's	7:30	5:15	8,250

[illegible]

[illegible]

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]